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(54) Title: USE OF ENZYME FOR CLEANING

(57) Abstract: The present invention relates to the use of an enzyme composition for cleaning in place.

Use of Enzyme for Cleaning

Field of the Invention

The present invention relates to the use of an enzyme having proteolytic activity for cleaning-in-place of process or production equipment, a detergent composition comprising the enzyme and a method for cleaning-in-place.

Description of the Related Art

Cleaning-in-place (CIP) is commonly used for cleaning storage tanks, bioreactors, fermenters, mix vessels, pipelines and other equipment used in biotech manufacturing, pharmaceutical manufacturing and food and beverage manufacturing.

Repeatable, reliable, and effective cleaning is of the utmost importance in a manufacturing facility because otherwise the subsequent production process may be contaminated with undesired micro-organism, which can spoil the production of for example food products.

In many industrial applications, such as the manufacture of foods and beverages, hard surfaces commonly become contaminated with soils such as carbohydrate, proteinaceous, and hardness soils, food oil soils, fat soils, and other soils. Such soils can arise from the manufacture of both liquid and solid foodstuffs. Carbohydrate soils, such as cellulosics, monosaccharides, disaccharides, oligosaccharides, starches, gums, and other complex materials, when dried, can form tough, hard to remove soils, particularly when combined with other soil components such as proteins, fats, oils, minerals, and others. The removal of such carbohydrate soils can be a significant problem. Similarly, other materials such as proteins, fats, and oils can also form hard to remove soil and residues.

Food and beverage soils are particularly tenacious when they are heated during processing. Foods and beverages are heated for a variety of reasons during processing. For example, in dairy plants, dairy products are heated on a pasteurizer (e.g., HTST-high temperature short-time-pasteurizer or UHT-ultra-high temperature-pasteurizer) in order to pasteurize the dairy product. In brewing, wort is boiled to breakdown the components of the grain into fermentable sugars. Also, many food and beverage products are concentrated or created as a result of evaporation.

Specific examples of food and beverage products that are concentrated using evaporators include dairy products such as whole and skimmed milk, condensed milk, whey and whey derivatives, buttermilk, proteins, lactose solutions, and lactic acid; protein solutions such as soya whey, nutrient yeast and fodder yeast, and whole egg; fruit juices such as orange and other citrus juices, apple juice and other pomaceous juices, red berry juice, coconut milk, and tropical fruit juices; vegetable juices such as tomato juice, beetroot juice, carrot juice, and grass

juice; starch products such as glucose, dextrose, fructose, isomerose, maltose, starch syrup, and dextrine; sugars such as liquid sugar, white refined sugar, sweetwater, and inulin; extracts such as coffee and tea extracts, hop extract, malt extract, yeast extract, pectin, and meat and bone extracts; hydrolyzates such as whey hydrolyzate, soup seasonings, milk hydrolyzate, and protein hydrolyzate; beer such as de-alcoholized beer and wort; and baby food, egg whites, bean oils, and fermented liquors.

CIP cleaning techniques are a specific cleaning regimen adapted for removing soils from the internal components of tanks, lines, pumps, and other production and process equipment used for processing typically liquid product streams such as beverages, milk, juices, etc. CIP cleaning involves passing cleaning solutions through the system without dismantling any system components. The minimum CIP technique involves passing/circulating the cleaning solution through the equipment and then resuming normal processing. Any product contaminated by cleaner residue can be discarded. Often CIP methods involve a first rinse, the application of the cleaning solutions, and a second rinse with potable water followed by resumed operations. The process can also include any other contacting step in which a rinse, acidic or basic functional fluid, solvent or other cleaning component such as hot water, cold water, etc. can be contacted with the equipment at any step during the process. Often the final potable water rinse is skipped in order to prevent contamination of the equipment with bacteria following the cleaning and/or sanitizing step.

Conventional CIP techniques, however, are not always sufficient at removing all types of soils. Specifically, it has been found that low density organic soils, e.g., ketchup, barbeque sauce, are not easily removed using traditional CIP cleaning techniques. Thermally degraded soils are also particularly difficult to remove using conventional CIP techniques.

Fermentation soil from brewing is another type of soil that is particularly difficult to remove from a surface. Brewing beer and wine requires the fermentation of sugars derived from starch-based material e.g., malted barley or fruit juice, e.g., grapes. Fermentation uses yeast to turn the sugars in wort or juice to alcohol and carbon dioxide. During fermentation, the wort becomes beer and the juice becomes wine. Once the boiled wort is cooled and placed in a fermenter, yeast and/or bacteria is propagated in the wort and it is left to ferment, which requires a week to months depending on the type of yeast or bacteria and style of the beer or wine. In addition to producing alcohol, fine particulate matter suspended in the wort settles during fermentation. Once fermentation is complete, the yeast also settles, leaving the beer or wine clear, but the fermentation tanks soiled with dead yeast cells, proteins, hop resins, and/or grape skins. Beer is a food product with is subject to microbiological spoilage. Breweries generally process a number of product types and must prevent “carryover” of one product into the next product. Cleaning of the fermentation is therefore very important.

Often during the fermentation process in commercial brewing, the fermentation tanks develop a ring of soil, i.e. brandhefe ring, which is particularly difficult to remove. Brandhefe rings are tough, tacky material composed of dried-up yeast, albumen, and hop resins. Traditional CIP methods of cleaning fermentation tanks do not always remove this soil. Thus, brewers often resort to climbing inside of the tanks and manually scrubbing them to remove the soil.

Moreover, traditional caustic cleaning methods necessitate fairly high temperatures for optimal cleaning Typical cleaning must be performed at temperatures of at least about 60-75°C. Therefore, the existing cleaning methods require the additional time and energy to sufficiently heat the food processing surface or washing vessel.

Furthermore, traditional CIP cleaning is performed in one of two ways with a caustic detergent composition typically composed of sodium hydroxide or with an acid-based detergent composition. Both traditional methods of CIP cleaning suffer from a number of setbacks. Acidic systems provide inferior cleaning and often are unable to adequately remove the aforementioned soils. This results in the need to expend greater time, energy, and effort to adequately clean the food processing surface. Alkaline cleaning systems are generally more effective at removing the soils; however, they suffer from problems of their own. Traditional caustic soda-based cleaning cannot be performed under high CO_2 conditions due to the risk of tank implosion caused by the removal of CO_2 by reaction with sodium hydroxide. Various types of food processing surfaces are often under an enriched CO_2 atmosphere. For example, in brewery applications this may be the result of intentionally creating the enriched CO_2 atmosphere to exclude oxygen from the vessel during fermentation or as a by-product of fermentation. When caustic soda is used under an enriched CO_2 atmosphere, it reacts with the CO_2, which results in substantial reduction in pressure. The change in pressure is so substantial that the tank will implode. Thus, in order to clean under alkaline conditions with caustic soda, the food processing surface must be vented to remove the CO_2. Adequate venting can take extensive amounts of time. This increases the amount of time that the food processing surface is soiled and not in condition to be used for its intended purpose, which is not time or cost effective. US patent application US2014/0261546 concerns a method for generating carbonate in situ in a use solution and using the solution for CIP of food processing surface. One problem associated with CIP is foaming during the cleaning process. The choice of surfactants used in the process is therefore important. A low foaming surfactant shoud be used as stated in US2014/0261546.

SUMMARY OF THE INVENTION

The present invention concerns the use of an enzyme having proteolytic activity for cleaning-in-place of process or production equipment

The invention further concerns a detergent composition comprising an enzyme having proteolytic activity, a carbonate source and water.

Further, the invention concerns a method for cleaning-in-place, wherein the enzyme having proteolytic activity is circulated in a process or production equipment.

DEFINITIONS

Benchmark cleaning: By the term "benchmark cleaning" is meant a cleaning of a manually soiled stainless steel discs as described in Assay I or a fermentation tank after fermentation of beer as described in Assay II, which benchmark cleaning is performed with 1% NaOH under the same conditions as described in the two assays.

Cleaning-in-place: "Cleaning-In-Place" or "CIP" is a method for cleaning the interior surfaces of process equipment or tanks without dismanteling the equipment. Process equipment can be processing tanks, storage tanks, pipelines, heat-exchangers, homogenizers, centrifuges, evaporators, extruders, coolers, storage tanks, sieves, hydroclones, filter units and filter membranes. CIP can also be used in road tankers transporting liquid food such as milk or beer, or in equipment used in slaughter houses.

Fermentation soil: During fermentation of beer yeast cells, proteins, hops and other particulate matter settles in the process or production equipment and leaves the surface of the equipment soiled with dead yeast cells, protein, hop and other particulate matter. The soil that is developed during fermentation of beer settles as a ring of soil, also known as brandhefe ring, in the top of the tank. This soil ring is a sticky kind of material that is especially difficult to remove. In one embodiment of the invention, the fermentation soil consists of yeast cells, proteins, hops and other particulate material. In one embodiment of the invention the fermentation soil consists of yeast cells, proteins and hops. In one embodiment of the invention the fermentation soil consists of yeast cells and proteins.

Cleaning performance: The term "cleaning performance" is a measure for the reduction and/or removal of fermentation soil from the process and/or production equipment. Cleaning performance is determined either gravimetrically or visually as described in Assay I and Assay II. Gravimetric determination of cleaning performance is described in Assay I and is performed by weight determination of the removed soil and calculated as % effective soil removal in proportion to the total initial amount of soil. The results a finally normalized so that the cleaning performance of the benchmark cleaning, 1% NaOH, is set to 100. Visual determination of the cleaning performance is described in Assay II and is performed by visually rating of the degree of cleanliness of the production surface on a scale from 1-5, where 1 is clean, 2 is lightly soiled, 3 is soiled, 4 is very soiled and 5 is heavily soiled.

Enzyme having proteolytic activity: The term "enzyme having proteolytic activity" means an enzyme acting on peptide bonds in proteins. The terms "enzyme having proteolytic activity" and "protease" are used interchangeably.

Enzyme having amylolytic activity: The term "enzyme having amylolytic activity" means the activity of alpha-1,4-glucan-4-glucanohydrolases (E.C. 3.2.1.1), which constitute a group of enzymes, catalyzing hydrolysis of starch and other linear and branched 1,4-glucosidic oligo- and polysaccharides. The terms "enzyme having amylolytic activity" and "amylase" or "alpha-amylase" are used interchangeably.

Enzyme having cellulolytic activity: The term "enzyme having cellulolytic activity" means enzymes capable of hydrolysing beta-1,4-glucosidic bonds in cellulose. In one embodiment of the invention the enzyme having cellulolytic activity include endo-1,4-beta-D-glucanases such as beta-1,4-glucanases, beta-1,4-endoglucan hydrolases, endoglucanase D, 1,4-(1,3,1,4)-beta-D-glucan 4-glucanohydrolases. In one embodiment the enzyme having cellulolytic activity is an endo-1,4-beta-D-glucanase as defined in SEQ ID NO: 6. In one embodiment the enzyme having cellulolytic activity is an endo-1,4-beta-D-glucanase derived from *Trichoderma reesei.*

Sequence identity: The relatedness between two amino acid sequences or between two nucleotide sequences is described by the parameter "sequence identity".

For purposes of the present invention, the sequence identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *Trends Genet.* 16: 276-277), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labeled "longest identity" (obtained using the –nobrief option) is used as the percent identity and is calculated as follows:

(Identical Residues x 100)/(Length of Alignment – Total Number of Gaps in Alignment)

For purposes of the present invention, the sequence identity between two deoxyribonucleotide sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *supra*) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *supra*), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EDNAFULL (EMBOSS version of NCBI NUC4.4) substitution matrix. The output of Needle labeled "longest identity" (obtained using the –nobrief option) is used as the percent identity and is calculated as follows:

(Identical Deoxyribonucleotides x 100)/(Length of Alignment – Total Number of Gaps in Alignment).

Variant: The term "variant" means a polypeptide having a specific activity comprising an alteration, *i.e.*, a substitution, insertion, and/or deletion, at one or more (*e.g.*, several) positions. A substitution means replacement of the amino acid occupying a position with a different amino acid; a deletion means removal of the amino acid occupying a position; and an insertion means adding an amino acid adjacent to and immediately following the amino acid occupying a position. In describing the variants of the present invention, the nomenclature described below is adapted for ease of reference. The accepted IUPAC single letter or three letter amino acid abbreviation is employed.

Substitutions. For an amino acid substitution, the following nomenclature is used: Original amino acid, position, substituted amino acid. Accordingly, the substitution of threonine at position 226 with alanine is designated as "Thr226Ala" or "T226A". Multiple mutations are separated by addition marks ("+"), *e.g.*, "Gly205Arg + Ser411Phe" or "G205R + S411F", representing substitutions at positions 205 and 411 of glycine (G) with arginine (R) and serine (S) with phenylalanine (F), respectively.

Deletions. For an amino acid deletion, the following nomenclature is used: Original amino acid, position, *. Accordingly, the deletion of glycine at position 195 is designated as "Gly195*" or "G195*". Multiple deletions are separated by addition marks ("+"), *e.g.*, "Gly195* + Ser411*" or "G195* + S411*".

Insertions. For an amino acid insertion, the following nomenclature is used: Original amino acid, position, original amino acid, inserted amino acid. Accordingly the insertion of lysine after glycine at position 195 is designated "Gly195GlyLys" or "G195GK". An insertion of multiple amino acids is designated [Original amino acid, position, original amino acid, inserted amino acid #1, inserted amino acid #2; etc.]. For example, the insertion of lysine and alanine after glycine at position 195 is indicated as "Gly195GlyLysAla" or "G195GKA".

In such cases, the inserted amino acid residue(s) are numbered by the addition of lower case letters to the position number of the amino acid residue preceding the inserted amino acid residue(s). In the above example, the sequence would thus be:

Parent:	Variant:
195	195 195a 195b
G	G - K - A

Multiple alterations. Variants comprising multiple alterations are separated by addition marks ("+"), *e.g.*, "Arg170Tyr+Gly195Glu" or "R170Y+G195E" representing a substitution of arginine and glycine at positions 170 and 195 with tyrosine and glutamic acid, respectively.

Different alterations. Where different alterations can be introduced at a position, the different alterations are separated by a comma, *e.g.*, "Arg170Tyr,Glu" represents a substitution

of arginine at position 170 with tyrosine or glutamic acid. Thus, "Tyr167Gly,Ala + Arg170Gly,Ala" designates the following variants:

"Tyr167Gly+Arg170Gly", "Tyr167Gly+Arg170Ala", "Tyr167Ala+Arg170Gly", and "Tyr167Ala+Arg170Ala".

Wash liquor: The term "wash liquor" is defined herein as a solution or mixture of a carbonate source and water. The wash liquor can optionally comprise a surfactant and/or an enzyme having proteolytic activity.

The wash liquor can further comprise detergent ingredients such as calcium chloride, sodium formate, sorbitol, glycerol and mono propylene glycol, chelators and anti-/defoaming agents.

DETAILED DESCRIPTION OF THE INVENTION

The present invention concerns the use of an enzyme for cleaning-in-place of process or production equipment, wherein the enzyme is selected from the group consisting of:

a. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus licheniformis*,
b. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus halodurans*,
c. an enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1; and
d. an enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3.

The inventor has surprisingly found that the use of the specific enzymes having proteolytic activity has a good cleaning performance without foaming.

In one embodiment of the invention, the process or production equipment is used in the food industry, pharma industry and biotech industry and can be cleaned with the enzyme having proteolytic activity. Especially, process or production equipment used in the brewing industry, dairy industry, pharma industry in biotech industry.

In one embodiment of the invention, the enzyme is especially good for cleaning process or production equipment for brewing, such as fermentation tanks for fermentation of the beer or wine. When the specific enzyme having proteolytic activity is used, then it is not necessary to use harsh chemicals such as sodium hydroxide. This makes it safer for the persons handling

the chemicals/detergents for the CIP process and no harsh chemicals are discharged with the waste water.

For fermentation tanks (fermentation vessels or brewing wessels) it is of particular relevance that the vessel is microbiologically clean after going through a cleaning process so the next brew is not contaminated with undesired microorganisms. The basic ingredients of beer are water and a starch source, such as malted barley, which is able to be fermented by the use of brewers yeast. After fermenting, the brewing vessel is soiled with residues of yeast cells, tannin, sugar, scale, protein, oxidation products and the like. This fermentation soil can be difficult to remove from the vessel. In one embodiment of the invention the fermentation soil consists of yeast cells, proteins, hops and other particulate material. In one embodiment of the invention, the fermentation soil consists of yeast cells, proteins and hops. In one embodiment of the invention, the fermentation soil consists of yeast cells and proteins.

The inventor has surprisingly found that the use of an enzyme having proteolytic activity cleans such vessel by reducing or removing fermentation soil. By using the enzyme, the cleaning performance is comparable to the traditional cleaning with NaOH (benchmark cleaning) and cleaning with harsh chemicals can thereby be omitted. The inventor has found that when using the present enzyme the amount of foam formed during cleaning is significantly lower than when CIP cleaning with other enzymes having proteolytic activity such as Savinase™ (SEQ ID NO: 2) which is an enzyme derived from *Bacillus clausii*. The claimed enzymes having proteolytic activity has low foaming properties which is utmost importance when used for CIP. Low foaming properties means that the enzyme generates less foam than the amount of foam generaged when CIP cleaning with Savinase™. In one embodiment, the enzyme having proteolytic activity generates at least 10% less foam than generated by Savinase™ under same cleaning conditions and measured by volume. In one embodiment the enzyme generates at least 20% less foam, at least 30% less foam, at least 40% less foam or at least 50% less foam.

In one embodiment of the invention, the enzyme having proteolytic activity is derived from *Bacillus licheniformis*. In one embodiment, the enzyme is derived from *Bacillus halodurans*.

In one embodiment, the enzyme has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1.

In one embodiment, the enzyme has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3

The cleaning-in-place can be further improved by using one or more enzyme in combination with the enzyme having proteolytic activity. The one ore more further enzymes may be selected from the group consisting of hemicellulases, peroxidases, proteases, cellulases, xylanases, lipases, phospholipases, esterases, cutinases, pectinases, mannanases, pectate

lyases, keratinases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, pullulanases, tannases, pentosanases, malanases, ß-glucanases, arabinosidases, hyaluronidase, chondroitinase, laccase, chlorophyllases, amylases, perhydrolases, peroxidases or mixtures thereof.

In one embodiment of the invention, an enzyme having amylolytic activity is used. In one embodiment an enzyme having cellulolytic activity is used.

The enzyme having amylolytic activity is selected from the group consisting of:

a. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and
b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

The enzyme having cellulolytic activity is selected from the group consisting of:

a. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 6;
b. an enzyme derived from *Humicola insolens,* and
c. an enzyme derived from *Trichoderma reesei*.

In one embodiment of the invention, the enzyme having proteolytic activity is derived from *Bacillus licheniformis*, and is used together with an enzyme having amylolytic activity selected from the group consisting of:

a. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and
b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

In one embodiment, the enzyme having proteolytic activity is derived from *Bacillus licheniformis*, and is used together with an enzyme having cellulolytic activity selected from the group consisting of:

a. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 6;
b. an enzyme derived from *Humicola insolens*; and
c. an enzyme derived from *Trichoderma reesei*.

In one embodiment, the enzyme having proteolytic activity is derived from *Bacillus halodurans*, and is used together with an enzyme having amylolytic activity selected from the group consisting of:

a. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and
b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

In one embodiment, the enzyme having proteolytic activity is derived from *Bacillus halodurans*, and is used together with an enzyme having cellulolytic activity selected from the group consisting of:

a. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 6;
b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 8; and
c. an enzyme derived from *Trichoderma reesei*.

In one embodiment, the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having amylolytic activity selected from the group consisting of:

a. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and
b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%,

at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

In one embodiment, the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having cellulolytic activity selected from the group consisting of:

a. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 6;
b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 8; and
c. an enzyme derived from *Trichoderma reesei*.

In one embodiment, the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3 and is used together with an enzyme having amylolytic activity selected from the group consisting of:

a. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and
b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

In one embodiment, the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3, and is used together with an enzyme having cellulolytic activity selected from the group consisting of:

a. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 6;

b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 8; and

c. an enzyme derived from *Trichoderma reesei*.

In one embodiment, the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having amylolytic activity having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and an enzyme having cellulolytic activity having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 6.

In one embodiment, the the enzyme having proteolytic activity has 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having amylolytic activity having 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and an enzyme having cellulolytic activity having 100% sequence identity to SEQ ID NO: 6.

During the cleaning-in-place the enzyme composition may be used together with other cleaning compounds such as at least one surfactant, at least one surfactant system, at least one soap or any mixtures thereof. By combining the use of the enzyme composition with a further cleaning compound the cleaning performance may be further enhanced.

The surfactant should be selected carefully so a low foaming surfactant is used. Especially non-ionic surfactants, amphoteric surfactant or mixtures thereof can be used.

The non-ionic surfactant can be selected from the group consisting of glycerol derivatives, sorbitan, glucose, sucrose derivatives, fatty acid ethoxylates, fatty acid ethoxylates propoxylates, fatty alcohol ethoxylates, alkyl phenol ethoxylates, fatty alcohol ethoxylates propoxylates, fatty esters of polyalcohol ethoxylates, end-blocked ethoxylates, polypropylene glycols and polyethylene glycols.

The amphoteric surfactant is selected from alkylimidazoline, alkylbetaines, alkylamidobetaines and protein derivatives.

The cleaning performacence on fermentation soil is comparable to benchmark cleaning. when the enzyme having proteolytic activity is used.

One way of measuring the cleaning performance is using Assay I. The inventor has found that the cleaning performance on fermentation soil is comparable to benchmark cleaning when measured with Assay I.

Another way of measuring the cleaing performance is using Assay II. The inventor has found that the cleaning performance on fermentation soil is comparable to benchmark cleaning when measured with Assay II.

The enzyme having proteolytic activity may be used for CIP at a temperature within the range of 10-30°C, such as in the range of 10-25°C, such as in the range of 15-25°C, in the range of 15-20°C or in the range of 18-22°C.

The invention further concerns detergent composition for cleaning-in-place which detergent composition comprises:

a. From 0.001 wt. % enzyme protein to 0.16 wt. % enzyme protein of an enzyme having proteolytic activity, which enzyme is selected from the group consisting of:
b. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus licheniformis*;
c. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus halodurans*;
d. an enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1; and
e. an enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3;
f. optionally from 0.01 wt. % to 2 wt. % of a surfactant;
g. from 0.5 wt% to 2 wt% of a carbonate source; and
h. water.

The composition may comprise an enzyme having proteolytic activity where the enzyme is derived from *Bacillus licheniformis*. The composition may comprise an enzyme having proteolytic activity, where the enzyme is derived from *Bacillus halodurans*. The composition may comprise an enzyme having proteolytic activity, where the enzyme has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1. The composition may comprise an enzyme having proteolytic activity where the enzyme has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3

The composition can further comprise one or more enzymes selected from: hemicellulases, peroxidases, proteases, cellulases, xylanases, lipases, phospholipases, esterases, cutinases, pectinases, mannanases, pectate lyases, keratinases, reductases,

oxidases, phenoloxidases, lipoxygenases, ligninases, pullulanases, tannases, pentosanases, malanases, ß-glucanases, arabinosidases, hyaluronidase, chondroitinase, laccase, chlorophyllases, amylases, perhydrolases, peroxidases or mixtures thereof.

In general, the properties of the selected enzyme(s) should be compatible with the selected detergent, (*i.e.*, pH-optimum, compatibility with other enzymatic and non-enzymatic ingredients, etc.), and the enzyme(s) should be present in effective amounts.

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

Especially suitable cellulases are the alkaline or neutral cellulases having colour care benefits. Examples of such cellulases are cellulases described in EP 0 495 257, EP 0 531 372, WO 96/11262, WO 96/29397, WO 98/08940. Other examples are cellulase variants such as those described in WO 94/07998, EP 0 531 315, US 5,457,046, US 5,686,593, US 5,763,254, WO 95/24471, WO 98/12307 and WO99/001544.

Other cellulases are endo-beta-1,4-glucanase enzyme having a sequence of at least 97% identity to the amino acid sequence of position 1 to position 773 of SEQ ID NO:2 of WO 2002/099091 or a family 44 xyloglucanase, which a xyloglucanase enzyme having a sequence of at least 60% identity to positions 40-559 of SEQ ID NO: 2 of WO 2001/062903.

Commercially available cellulases include Celluzyme™, and Carezyme™ (Novozymes A/S) Carezyme Premium™ (Novozymes A/S), Celluclean ™ (Novozymes A/S), Celluclean Classic™ (Novozymes A/S), Cellusoft™ (Novozymes A/S), Whitezyme™ (Novozymes A/S), Clazinase™, and Puradax HA™ (Genencor International Inc.), and KAC-500(B)™ (Kao Corporation).

Suitable mannanases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included. The mannanase may be an alkaline mannanase of Family 5 or 26. It may be a wild-type from *Bacillus* or *Humicola*, particularly *B. agaradhaerens, B. licheniformis, B. halodurans, B. clausii*, or *H. insolens*. Suitable mannanases are described in WO 1999/064619. A commercially available mannanase is Mannaway (Novozymes A/S).

Suitable proteases include those of bacterial, fungal, plant, viral or animal origin e.g. vegetable or microbial origin. Microbial origin is preferred. Chemically modified or protein engineered mutants are included. It may be an alkaline protease, such as a serine protease or a metalloprotease. A serine protease may for example be of the S1 family, such as trypsin, or the S8 family such as subtilisin. A metalloproteases protease may for example be a thermolysin from e.g. family M4 or other metalloprotease such as those from M5, M7 or M8 families.

The term "subtilases" refers to a sub-group of serine protease according to Siezen et al., Protein Engng. 4 (1991) 719-737 and Siezen et al. Protein Science 6 (1997) 501-523. Serine proteases are a subgroup of proteases characterized by having a serine in the active site, which forms a covalent adduct with the substrate. The subtilases may be divided into 6 sub-divisions, i.e. the Subtilisin family, the Thermitase family, the Proteinase K family, the Lantibiotic peptidase family, the Kexin family and the Pyrolysin family.

Examples of subtilases are those derived from *Bacillus* such as *Bacillus lentus*, *B. alkalophilus*, *B. subtilis*, *B. amyloliquefaciens*, *Bacillus pumilus* and *Bacillus gibsonii* described in; US7262042 and WO09/021867, and *subtilisin lentus*, *subtilisin Novo*, *subtilisin Carlsberg*, *Bacillus licheniformis*, *subtilisin BPN'*, *subtilisin 309*, *subtilisin 147* and *subtilisin 168* described in WO89/06279 and protease PD138 described in (WO93/18140). Other useful proteases may be those described in WO92/175177, WO01/016285, WO02/026024 and WO02/016547. Examples of trypsin-like proteases are trypsin (e.g. of porcine or bovine origin) and the *Fusarium* protease described in WO89/06270, WO94/25583 and WO05/040372, and the chymotrypsin proteases derived from *Cellumonas* described in WO05/052161 and WO05/052146.

A further preferred protease is the alkaline protease from *Bacillus lentus* DSM 5483, as described for example in WO95/23221, and variants thereof which are described in WO92/21760, WO95/23221, EP1921147 and EP1921148.

Examples of metalloproteases are the neutral metalloprotease as described in WO07/044993 (Genencor Int.) such as those derived from *Bacillus amyloliquefaciens*.

Examples of useful proteases are the variants described in: WO92/19729, WO96/034946, WO98/20115, WO98/20116, WO99/011768, WO01/44452, WO03/006602, WO04/03186, WO04/041979, WO07/006305, WO11/036263, WO11/036264, especially the variants with substitutions in one or more of the following positions: 3, 4, 9, 15, 27, 36, 57, 68, 76, 87, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 106, 118, 120, 123, 128, 129, 130, 160, 167, 170, 194, 195, 199, 205, 206, 217, 218, 222, 224, 232, 235, 236, 245, 248, 252 and 274 using the BPN' numbering. More preferred the subtilase variants may comprise the mutations: S3T, V4I, S9R, A15T, K27R, *36D, V68A, N76D, N87S,R, *97E, A98S, S99G,D,A, S99AD, S101G,M,R S103A, V104I,Y,N, S106A, G118V,R, H120D,N, N123S, S128L, P129Q, S130A, G160D, Y167A, R170S, A194P, G195E, V199M, V205I, L217D, N218D, M222S, A232V, K235L, Q236H, Q245R, N252K, T274A (using BPN' numbering).

Suitable commercially available protease enzymes include those sold under the trade names Alcalase®, Duralase™, Durazym™, Relase®, Relase® Ultra, Savinase®, Savinase® Ultra, Primase®, Polarzyme®, Kannase®, Liquanase®, Liquanase® Ultra, Ovozyme®, Coronase®, Coronase® Ultra, Neutrase®, Everlase® and Esperase® (Novozymes A/S), those sold under the tradename Maxatase®, Maxacal®, Maxapem®, Purafect®, Purafect Prime®, ,

Purafect MA®, Purafect Ox®, Purafect OxP®, Puramax®, Properase®, , FN2®, FN3® , FN4®, Excellase®, Eraser®, Opticlean® and Optimase® (Danisco/DuPont), Axapem™ (Gist-Brocases N.V.), BLAP (sequence shown in Figure 29 of US5352604) and variants hereof (Henkel AG) and KAP (*Bacillus alkalophilus* subtilisin) from Kao.

Suitable lipases and cutinases include those of bacterial or fungal origin. Chemically modified or protein engineered mutant enzymes are included. Examples include lipase from *Thermomyces*, e.g. from *T. lanuginosus* (previously named *Humicola lanuginosa*) as described in EP258068 and EP305216, cutinase from *Humicola*, e.g. *H. insolens* (WO96/13580), lipase from strains of *Pseudomonas* (some of these now renamed to *Burkholderia*), e.g. *P. alcaligenes* or *P. pseudoalcaligenes* (EP218272), *P. cepacia* (EP331376), *P. sp.* strain SD705 (WO95/06720 & WO96/27002), *P. wisconsinensis* (WO96/12012), GDSL-type *Streptomyces* lipases (WO10/065455), cutinase from *Magnaporthe grisea* (WO10/107560), cutinase from *Pseudomonas mendocina* (US5,389,536), lipase from *Thermobifida fusca* (WO11/084412), *Geobacillus stearothermophilus* lipase (WO11/084417), lipase from *Bacillus subtilis* (WO11/084599), and lipase from *Streptomyces griseus* (WO11/150157) and *S. pristinaespiralis* (WO12/137147).

Other examples are lipase variants such as those described in EP407225, WO92/05249, WO94/01541, WO94/25578, WO95/14783, WO95/30744, WO95/35381, WO95/22615, WO96/00292, WO97/04079, WO97/07202, WO00/34450, WO00/60063, WO01/92502, WO07/87508 and WO09/109500.

Preferred commercial lipase products include include Lipolase™, Lipex™; Lipolex™ and Lipoclean™ (Novozymes A/S), Lumafast (originally from Genencor) and Lipomax (originally from Gist-Brocades).

Still other examples are lipases sometimes referred to as acyltransferases or perhydrolases, e.g. acyltransferases with homology to *Candida antarctica* lipase A (WO10/111143), acyltransferase from *Mycobacterium smegmatis* (WO05/56782), perhydrolases from the CE 7 family (WO09/67279), and variants of the *M. smegmatis* perhydrolase in particular the S54V variant used in the commercial product Gentle Power Bleach from Huntsman Textile Effects Pte Ltd (WO10/100028).

Suitable amylases which can be used together with the detergent composition of the invention may be an alpha-amylase or a glucoamylase and may be of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, alpha-amylases obtained from *Bacillus*, *e.g.*, a special strain of *Bacillus licheniformis*, described in more detail in GB 1,296,839.

Suitable amylases include amylases having SEQ ID NO: 2 in WO 95/10603 or variants having 90% sequence identity to SEQ ID NO: 3 thereof. Preferred variants are described in WO 94/02597, WO 94/18314, WO 97/43424 and SEQ ID NO: 4 of WO 99/019467, such as variants

with substitutions in one or more of the following positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 178, 179, 181, 188, 190, 197, 201, 202, 207, 208, 209, 211, 243, 264, 304, 305, 391, 408, and 444.

Different suitable amylases include amylases having SEQ ID NO: 6 in WO 02/010355 or variants thereof having 90% sequence identity to SEQ ID NO: 6. Preferred variants of SEQ ID NO: 6 are those having a deletion in positions 181 and 182 and a substitution in position 193.

Other amylases which are suitable are hybrid alpha-amylase comprising residues 1-33 of the alpha-amylase derived from *B. amyloliquefaciens* shown in SEQ ID NO: 6 of WO 2006/066594 and residues 36-483 of the *B. licheniformis* alpha-amylase shown in SEQ ID NO: 4 of WO 2006/066594 or variants having 90% sequence identity thereof. Preferred variants of this hybrid alpha-amylase are those having a substitution, a deletion or an insertion in one of more of the following positions: G48, T49, G107, H156, A181, N190, M197, I201, A209 and Q264. Most preferred variants of the hybrid alpha-amylase comprising residues 1-33 of the alpha-amylase derived from *B. amyloliquefaciens* shown in SEQ ID NO: 6 of WO 2006/066594 and residues 36-483 of SEQ ID NO: 4 are those having the substitutions:

M197T;

H156Y+A181T+N190F+A209V+Q264S; or

G48A+T49I+G107A+H156Y+A181T+N190F+I201F+A209V+Q264S.

Further amylases which are suitable are amylases having SEQ ID NO: 6 in WO 99/019467 or variants thereof having 90% sequence identity to SEQ ID NO: 6. Preferred variants of SEQ ID NO: 6 are those having a substitution, a deletion or an insertion in one or more of the following positions: R181, G182, H183, G184, N195, I206, E212, E216 and K269. Particularly preferred amylases are those having deletion in positions R181 and G182, or positions H183 and G184.

Additional amylases which can be used are those having SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 2 or SEQ ID NO: 7 of WO 96/023873 or variants thereof having 90% sequence identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 7. Preferred variants of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 7 are those having a substitution, a deletion or an insertion in one or more of the following positions: 140, 181, 182, 183, 184, 195, 206, 212, 243, 260, 269, 304 and 476, using SEQ ID 2 of WO 96/023873 for numbering. More preferred variants are those having a deletion in two positions selected from 181, 182, 183 and 184, such as 181 and 182, 182 and 183, or positions 183 and 184. Most preferred amylase variants of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 7 are those having a deletion in positions 183 and 184 and a substitution in one or more of positions 140, 195, 206, 243, 260, 304 and 476.

Other amylases which can be used are amylases having SEQ ID NO: 2 of WO 08/153815, SEQ ID NO: 10 in WO 01/66712 or variants thereof having 90% sequence identity

to SEQ ID NO: 2 of WO 08/153815 or 90% sequence identity to SEQ ID NO: 10 in WO 01/66712. Preferred variants of SEQ ID NO: 10 in WO 01/66712 are those having a substitution, a deletion or an insertion in one of more of the following positions: 176, 177, 178, 179, 190, 201, 207, 211 and 264.

Further suitable amylases are amylases having SEQ ID NO: 2 of WO 09/061380 or variants having 90% sequence identity to SEQ ID NO: 2 thereof. Preferred variants of SEQ ID NO: 2 are those having a truncation of the C-terminus and/or a substitution, a deletion or an insertion in one of more of the following positions: Q87, Q98, S125, N128, T131, T165, K178, R180, S181, T182, G183, M201, F202, N225, S243, N272, N282, Y305, R309, D319, Q320, Q359, K444 and G475. More preferred variants of SEQ ID NO: 2 are those having the substitution in one of more of the following positions: Q87E,R, Q98R, S125A, N128C, T131I, T165I, K178L, T182G, M201L, F202Y, N225E,R, N272E,R, S243Q,A,E,D, Y305R, R309A, Q320R, Q359E, K444E and G475K and/or deletion in position R180 and/or S181 or of T182 and/or G183. Most preferred amylase variants of SEQ ID NO: 2 are those having the substitutions:

N128C+K178L+T182G+Y305R+G475K;

N128C+K178L+T182G+F202Y+Y305R+D319T+G475K;

S125A+N128C+K178L+T182G+Y305R+G475K; or

S125A+N128C+T131I+T165I+K178L+T182G+Y305R+G475K wherein the variants are C-terminally truncated and optionally further comprises a substitution at position 243 and/or a deletion at position 180 and/or position 181.

Further suitable amylases are amylases having SEQ ID NO: 1 of WO13184577 or variants having 90% sequence identity to SEQ ID NO: 1 thereof. Preferred variants of SEQ ID NO: 1 are those having a substitution, a deletion or an insertion in one of more of the following positions: K176, R178, G179, T180, G181, E187, N192, M199, I203, S241, R458, T459, D460, G476 and G477. More preferred variants of SEQ ID NO: 1 are those having the substitution in one of more of the following positions: K176L, E187P, N192FYH, M199L, I203YF, S241QADN, R458N, T459S, D460T, G476K and G477K and/or deletion in position R178 and/or S179 or of T180 and/or G181. Most preferred amylase variants of SEQ ID NO: 1 are those having the substitutions:

E187P+I203Y+G476K

E187P+I203Y+R458N+T459S+D460T+G476K,

wherein the variants optionally further comprises a substitution at position 241 and/or a deletion at position 178 and/or position 179.

Further suitable amylases are amylases having SEQ ID NO: 1 of WO10104675 or variants having 90% sequence identity to SEQ ID NO: 1 thereof. Preferred variants of SEQ ID NO: 1 are those having a substitution, a deletion or an insertion in one of more of the following

positions: N21, D97, V128 K177, R179, S180, I181, G182, M200, L204, E242, G477 and G478.

More preferred variants of SEQ ID NO: 1 are those having the substitution in one of more of the following positions: N21D, D97N, V128I K177L, M200L, L204YF, E242QA, G477K and G478K and/or deletion in position R179 and/or S180 or of I181 and/or G182. Most preferred amylase variants of SEQ ID NO: 1 are those having the substitutions:

N21D+D97N+V128I

wherein the variants optionally further comprises a substitution at position 200 and/or a deletion at position 180 and/or position 181.

Other suitable amylases are the alpha-amylase having SEQ ID NO: 12 in WO01/66712 or a variant having at least 90% sequence identity to SEQ ID NO: 12. Preferred amylase variants are those having a substitution, a deletion or an insertion in one of more of the following positions of SEQ ID NO: 12 in WO01/66712: R28, R118, N174; R181, G182, D183, G184, G186, W189, N195, M202, Y298, N299, K302, S303, N306, R310, N314; R320, H324, E345, Y396, R400, W439, R444, N445, K446, Q449, R458, N471, N484. Particular preferred amylases include variants having a deletion of D183 and G184 and having the substitutions R118K, N195F, R320K and R458K, and a variant additionally having substitutions in one or more position selected from the group: M9, G149, G182, G186, M202, T257, Y295, N299, M323, E345 and A339, most preferred a variant that additionally has substitutions in all these positions.

Other examples are amylase variants such as those described in WO2011/098531, WO2013/001078 and WO2013/001087.

Commercially available amylases are Duramyl™, Termamyl™, Fungamyl™, Stainzyme ™, Stainzyme Plus™, Natalase™, Liquozyme X and BAN™ (from Novozymes A/S), and Rapidase™ , Purastar™/Effectenz™, Powerase, Preferenz S1000, Preferenz S100 and Preferenz S110 (from Genencor International Inc./DuPont).

A peroxidase according to the invention is a peroxidase enzyme comprised by the enzyme classification EC 1.11.1.7, as set out by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB), or any fragment derived therefrom, exhibiting peroxidase activity.

Suitable peroxidases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful peroxidases include peroxidases from *Coprinopsis*, *e.g.*, from *C. cinerea* (EP 179,486), and variants thereof as those described in WO 93/24618, WO 95/10602, and WO 98/15257.

A peroxidase according to the invention also includes a haloperoxidase enzyme, such as chloroperoxidase, bromoperoxidase and compounds exhibiting chloroperoxidase or bromoperoxidase activity. Haloperoxidases are classified according to their specificity for halide ions. Chloroperoxidases (E.C. 1.11.1.10) catalyze formation of hypochlorite from chloride ions.

In an embodiment, the haloperoxidase of the invention is a chloroperoxidase. Preferably, the haloperoxidase is a vanadium haloperoxidase, *i.e.*, a vanadate-containing haloperoxidase. In a preferred method of the present invention the vanadate-containing haloperoxidase is combined with a source of chloride ion.

Haloperoxidases have been isolated from many different fungi, in particular from the fungus group dematiaceous hyphomycetes, such as *Caldariomyces*, *e.g.*, *C. fumago*, *Alternaria*, *Curvularia*, *e.g.*, *C. verruculosa* and *C. inaequalis*, *Drechslera*, *Ulocladium* and *Botrytis*.

Haloperoxidases have also been isolated from bacteria such as *Pseudomonas*, *e.g.*, *P. pyrrocinia* and *Streptomyces*, *e.g.*, *S. aureofaciens*.

In a preferred embodiment, the haloperoxidase is derivable from *Curvularia* sp., in particular *Curvularia verruculosa* or *Curvularia inaequalis*, such as *C. inaequalis* CBS 102.42 as described in WO 95/27046; or *C. verruculosa* CBS 147.63 or *C. verruculosa* CBS 444.70 as described in WO 97/04102; or from *Drechslera hartlebii* as described in WO 01/79459, *Dendryphiella salina* as described in WO 01/79458, *Phaeotrichoconis crotalarie* as described in WO 01/79461, or *Geniculosporium* sp. as described in WO 01/79460.

An oxidase according to the invention include, in particular, any laccase enzyme comprised by the enzyme classification EC 1.10.3.2, 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 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*, *Coprinopsis*, *e.g.*, *C. cinerea*, *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. radiata* (WO 92/01046), or *Coriolus*, *e.g.*, *C. hirsutus* (JP 2238885).

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

A laccase derived from *Coprinopsis* or *Myceliophthora* is preferred; in particular a laccase derived from *Coprinopsis cinerea*, as disclosed in WO 97/08325; or from *Myceliophthora thermophila*, as disclosed in WO 95/33836.

Examples of useful peroxidases include peroxidases from *Coprinus*, *e.g.*, from *C. cinereus*, and variants thereof as those described in WO 93/24618, WO 95/10602, and WO 98/15257. Commercially available peroxidases include Guardzyme™ (Novozymes A/S).

The detergent enzyme(s) may be included in a detergent composition by adding separate additives containing one or more enzymes, or by adding a combined additive comprising all of these enzymes. A detergent additive of the invention, *i.e.*, a separate additive or a combined additive, can be formulated, for example, as a granulate, liquid, slurry, etc. Preferred detergent additive formulations are granulates, in particular non-dusting granulates, liquids, in particular stabilized liquids, or slurries.

Non-dusting granulates may be produced, *e.g.* as disclosed in US 4,106,991 and 4,661,452 and may optionally be coated by methods known in the art. Examples of waxy coating materials are polyethyleneglycol (PEG) with mean molar 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. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

In one embodiment, the composition comprises an enzyme having amylolytic activity. In one embodiment the composition comprises an enzyme having cellulolytic activity. The use of these enzymes together with the enzyme having proteolytic activity ensures that the fermentation soil is effectively removed and that the cleaning performance is comparable to benchmark cleaning.

The enzyme composition may comprise an enzyme having amylolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and

b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5;

The enzyme composition may comprise enzyme having cellulolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at

least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;

b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 8; and

c. an enzyme derived from *Trichoderma reesei*.

In one embodiment of the invention, the enzyme composition may comprise an enzyme having proteolytic activity derived from *Bacillus licheniformis* together with an enzyme having amylolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and

b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

In one embodiment, the enzyme composition may comprise an enzyme having proteolytic activity derived from *Bacillus licheniformis* together with an enzyme having cellulolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;

b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 8; and

c. an enzyme derived from *Trichoderma reesei*.

In one embodiment, the enzyme composition may comprise an enzyme having proteolytic activity derived from *Bacillus halodurans* together with an enzyme having amylolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and

b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at

least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

In one embodiment, the enzyme composition may comprise an enzyme having proteolytic activity derived from *Bacillus halodurans* together with an enzyme having cellulolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;
b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 8; and
c. an enzyme derived from *Trichoderma reesei*.

In one embodiment, the enzyme composition may comprise an enzyme having proteolytic activity having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1 and an enzyme having amylolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and
b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

In one embodiment, the enzyme composition may comprise an enzyme having proteolytic activity haivng at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and an enzyme having cellulolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;

b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 8; and

c. an enzyme derived from *Trichoderma reesei*.

In one embodiment, the enzyme composition may comprise an enzyme having proteolytic activity and having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3 and an enzyme having amylolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and

b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

In one embodiment, the enzyme composition may comprise an enzyme having proteolytic activity and having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3, and an enzyme having cellulolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;

b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 8; and

c. an enzyme derived from *Trichoderma reesei*.

In one embodiment, the enzyme composition may comprise an enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1 and an enzyme having amylolytic activity having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and an enzyme having

cellulolytic activity having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6.

In one embodiment, the enzyme composition may comprise an enzyme having proteolytic activity having 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and an enzyme having amylolytic activity having 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and an enzyme having cellulolytic activity having 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6. The use of these specific enzymes removes the fermentation soil is effectively as shown in examples 1 and 2 and has a cleaning performance comparable to benchmark cleaning.

The detergent composition comprises a carbonate source, which may be an aquous solution of carbonic acid, carbonate and/or bicarbonate optionally generated in situ for example by forming a carbonate use solution as described in patent application US2014/0261546 (Ecolab) in paragraphs [0127] to [137] by use of the suitable sources described in paragraph [0059]. When a carbonate source is used together with the enzyme having proteolytic activity, the cleaning performance is comparable to benchmark cleaning and the time for cleaning is considerably shorter as it is not necessary to vent the equipment.

Examples of suitable alkaline sources of the detergent composition include, but are not limited to carbonate-based alkalinity sources, including, for example, carbonate salts such as alkali metal carbonates; caustic-based alkalinity sources, including, for example, alkali metal hydroxides; other suitable alkalinity sources may include metal silicate, metal borate, and organic alkalinity sources. Exemplary alkali metal carbonates that can be used include, but are not limited to, sodium carbonate, potassium carbonate, bicarbonate, sesquicarbonate, and mixtures thereof. Exemplary alkali metal hydroxides that can be used include, but are not limited to sodium, lithium, or potassium hydroxide. Exemplary metal silicates that can be used include, but are not limited to, sodium or potassium silicate or metasilicate. Exemplary metal borates include, but are not limited to, sodium or potassium borate. In addition to the first alkalinity source, the detergent composition and/or use solution may comprise a secondary alkalinity source. Examples of useful secondary alkaline sources include those described above.

In one embodiment of the invention, the composition comprises at least one surfactant, at least one surfactant system, at least one soap or any mixtures thereof. The use of a surfactant together with the enzyme having proteolytic activity gives a better cleaning performance.

The surfactant can be one or more non-ionic surfactants or one ore more amphoteric surfactant or a mixture thereof. The non-ionic surfactant can be selected from the group consisting of glycerol derivatives, sorbitan, glucose, sucrose derivatives, fatty acid ethoxylates, fatty acid ethoxylates propoxylates, fatty alcohol ethoxylates, alkyl phenol ethoxylates, fatty

alcohol ethoxylates propoxylates, fatty esters of polyalcohol ethoxylates, end-blocked ethoxylates, polypropylene glycols and polyethylene glycols.

The amphoteric surfactant is selected from alkylimidazoline, alkylbetaines, alkylamidobetaines and protein derivatives.

The detergent composition may contain about 0-10% by weight, such as about 1-5% of a detergent builder or co-builder, or a mixture thereof. The builder and/or co-builder may particularly be a chelating agent that forms water-soluble complexes with Ca and Mg. Any builder and/or co-builder known in the art for use in CIP detergents may be utilized. Non-limiting examples of builders include zeolites, diphosphates (pyrophosphates), triphosphates such as sodium triphosphate (STP or STPP), carbonates such as sodium carbonate, soluble silicates such as sodium metasilicate, layered silicates (*e.g.*, SKS-6 from Hoechst), ethanolamines such as 2-aminoethan-1-ol (MEA), diethanolamine (DEA, also known as 2,2'-iminodiethan-1-ol), triethanolamine (TEA, also known as 2,2',2"-nitrilotriethan-1-ol), and (carboxymethyl)inulin (CMI), and combinations thereof.

The detergent composition may also contain 0-10% by weight, such as about 5% of a detergent co-builder. The detergent composition may include a co-builder alone, or in combination with a builder, for example a zeolite builder. Non-limiting examples of co-builders include homopolymers of polyacrylates or copolymers thereof, such as poly(acrylic acid) (PAA) or copoly(acrylic acid/maleic acid) (PAA/PMA). Further non-limiting examples include citrate, chelators such as aminocarboxylates, aminopolycarboxylates and phosphonates, and alkyl- or alkenylsuccinic acid. Additional specific examples include 2,2',2"-nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), iminodisuccinic acid (IDS), ethylenediamine-*N*,*N*'-disuccinic acid (EDDS), methylglycinediacetic acid (MGDA), glutamic acid-*N*,*N*-diacetic acid (GLDA), 1-hydroxyethane-1,1-diphosphonic acid (HEDP), ethylenediaminetetra(methylenephosphonic acid) (EDTMPA), diethylenetriaminepentakis(methylenephosphonic acid) (DTMPA or DTPMPA), *N*-(2-hydroxyethyl)iminodiacetic acid (EDG), aspartic acid-*N*-monoacetic acid (ASMA), aspartic acid-*N*,*N*-diacetic acid (ASDA), aspartic acid-*N*-monopropionic acid (ASMP), iminodisuccinic acid (IDA), *N*-(2-sulfomethyl)-aspartic acid (SMAS), *N*-(2-sulfoethyl)-aspartic acid (SEAS), *N*-(2-sulfomethyl)-glutamic acid (SMGL), *N*-(2-sulfoethyl)-glutamic acid (SEGL), *N*-methyliminodiacetic acid (MIDA), α-alanine-*N*,*N*-diacetic acid (α-ALDA), serine-*N*,*N*-diacetic acid (SEDA), isoserine-*N*,*N*-diacetic acid (ISDA), phenylalanine-*N*,*N*-diacetic acid (PHDA), anthranilic acid-*N*,*N*-diacetic acid (ANDA), sulfanilic acid-*N*,*N*-diacetic acid (SLDA) , taurine-*N*,*N*-diacetic acid (TUDA) and sulfomethyl-*N*,*N*-diacetic acid (SMDA), *N*-(2-hydroxyethyl)ethylenediamine-*N*,*N'*,*N"*-triacetic acid (HEDTA), diethanolglycine (DEG), diethylenetriamine penta(methylenephosphonic acid) (DTPMP), aminotris(methylenephosphonic

acid) (ATMP), and combinations and salts thereof. Further exemplary builders and/or co-builders are described in, e.g., WO 09/102854, US 5977053.

The water for rinsing the process or production equipment may also include disinfecting agent. Examples of suitable disinfecting agents include compounds capable of liberating an active halogen species, such as Cl2, Br2, —OCl− and/or —OBr−, under conditions typically encountered during the cleansing process. Suitable disinfecting agents for use in the present method include, for example, chlorine-containing compounds such as a chlorine, a hypochlorite, and chloramine. Exemplary halogen-releasing compounds include the alkali metal dichloroisocyanurates, chlorinated trisodium phosphate, the alkali metal hypochlorites, monochloramine and dichloramine, and the like. Encapsulated chlorine sources may also be used to enhance the stability of the chlorine source in the composition (see, for example, U.S. Pat. Nos. 4,618,914 and 4,830,773, the disclosures of which are incorporated by reference herein for all purposes).

A disinfecting agent may also be a peroxygen or active oxygen source such as hydrogen peroxide, perborates, sodium carbonate peroxyhydrate, phosphate peroxyhydrates, potassium permonosulfate, and sodium perborate mono and tetrahydrate, with and without activators such as tetraacetylethylene diamine, and the like. The composition can include an effective amount of a bleaching agent. When the concentrate includes a bleaching agent, it can be included in an amount of about 0.1 wt. % to about 5 wt. % or from about 1 wt. % to about 2 wt. %.

Stabilizing agents that can be used in the detergent composition include, but are not limited to: primary aliphatic amines, betaines, borate, calcium ions, sodium citrate, citric acid, sodium formate, glycerine, malonic acid, organic diacids, polyols, propylene glycol, and mixtures thereof. The concentrate need not include a stabilizing agent, but when the concentrate includes a stabilizing agent, it can be included in an amount that provides the desired level of stability of the concentrate. Exemplary ranges of the stabilizing agent include up to about 10 wt. % or between about 0.5 wt. % to about 5 wt. %.

The compositions of the invention may optionally include a hydrotrope that aides in compositional stability and aqueous formulation. Functionally speaking, the suitable hydrotrope couplers which can be employed are non-toxic and retain the active ingredients in aqueous solution throughout the temperature range and concentration to which a concentrate or any use solution is exposed.

Any hydrotrope coupler may be used provided it does not react with the other components of the composition or negatively affect the performance properties of the composition. Representative classes of hydrotropic coupling agents or solubilizers which can be employed include anionic surfactants such as alkyl sulfates and alkane sulfonates, linear alkyl benzene or naphthalene sulfonates, secondary alkane sulfonates, alkyl ether sulfates or sulfonates, alkyl phosphates or phosphonates, dialkyl sulfosuccinic acid esters, sugar esters

(e.g., sorbitan esters), amine oxides (mono-, di-, or tri-alkyl) and C8-C10 alkyl glucosides. Preferred coupling agents for use in the present invention include n-octanesulfonate, available as NAS 8D from Ecolab Inc., n-octyl dimethylamine oxide, and the commonly available aromatic sulfonates such as the alkyl benzene sulfonates (e.g. xylene sulfonates) or naphthalene sulfonates, aryl or alkaryl phosphate esters or their alkoxylated analogues having 1 to about 40 ethylene, propylene or butylene oxide units or mixtures thereof. Other preferred hydrotropes include nonionic surfactants of C6-C24 alcohol alkoxylates (alkoxylate means ethoxylates, propoxylates, butoxylates, and co-or-terpolymer mixtures thereof) (preferably C6-C14 alcohol alkoxylates) having 1 to about 15 alkylene oxide groups (preferably about 4 to about 10 alkylene oxide groups); C6-C24 alkylphenol alkoxylates (preferably C8-C10 alkylphenol alkoxylates) having 1 to about 15 alkylene oxide groups (preferably about 4 to about 10 alkylene oxide groups); C6-C24 alkylpolyglycosides (preferably C6-C20 alkylpolyglycosides) having 1 to about 15 glycoside groups (preferably about 4 to about 10 glycoside groups); C6-C24 fatty acid ester ethoxylates, propoxylates or glycerides; and C4-C12 mono or dialkanolamides. A preferred hydrotope is sodium cumenesulfonate (SCS).

The composition of an optional hydrotrope can be present in the range of from about 0 to about 5 percent by weight.

Water conditioning agents function to inactivate water hardness and prevent calcium and magnesium ions from interacting with soils, surfactants, carbonate and hydroxide. Water conditioning agents therefore improve detergency and prevent long term effects such as insoluble soil redepositions, mineral scales and mixtures thereof. Water conditioning can be achieved by different mechanisms including sequestration, precipitation, ion-exchange and dispersion (threshold effect).

The water conditioning agents which can be used include inorganic water soluble water conditioning agents, inorganic water insoluble water conditioning agents, organic water soluble conditioning agents, and organic water insoluble water conditioning agents. Exemplary inorganic water soluble water conditioning agents include all physical forms of alkali metal, ammonium and substituted ammonium salts of carbonate, bicarbonate and sesquicarbonate; pyrophosphates, and condensed polyphosphates such as tripolyphosphate, trimetaphosphate and ring open derivatives; and, glassy polymeric metaphosphates of general structure Mn+2PnO3n+1 having a degree of polymerization n of from about 6 to about 21 in anhydrous or hydrated forms; and, mixtures thereof. Exemplary inorganic water insoluble water conditioning agents include aluminosilicate builders. Exemplary water soluble water conditioning agents include aminpolyacetates, polyphosphonates, aminopolyphosphonates, short chain carboxylates and polycarboxylates. Organic water soluble water conditioning agents useful in the compositions of the present invention include aminpolyacetates, polyphosphonates,

aminopolyphosphonates, short chain carboxylates and a wide variety of polycarboxylate compounds.

Aminopolyacetate water conditioning salts suitable for use herein include the sodium, potassium lithium, ammonium, and substituted ammonium salts of the following acids: ethylenediaminetetraacetic acid, N-(2-hydroxyethyl)-ethylenediamine triacetic acid, N-(2-hydroxyethyl)-nitrilodiacetic acid, diethylenetriaminepentaacetic acid, 1,2-diaminocyclohexanetetracetic acid and nitrilotriacetic acid; and, mixtures thereof. Polyphosphonates useful herein specifically include the sodium, lithium and potassium salts of ethylene diphosphonic acid; sodium, lithium and potassium salts of ethane-1-hydroxy-1,1-diphosphonic acid and sodium lithium, potassium, ammonium and substituted ammonium salts of ethane-2-carboxy-1,1-diphosphonic acid, hydroxymethanediphosphonic acid, carbonyldiphosphonic acid, ethane-1-hydroxy-1,1,2-triphosphonic acid, ethane-2-hydroxy-1,1,2-triphosphonic acid, propane-1,1,3,3-tetraphosphonic acid propane-1,1,2,3-tetraphophonic acid and propane 1,2,2,3-tetraphosphonic acid; and mixtures thereof. Examples of these polyphosphonic compounds are disclosed in British Pat. No. 1,026,366. For more examples see U.S. Pat. No. 3,213,030 to Diehl issued Oct. 19, 1965 and U.S. Pat. No. 2,599,807 to Bersworth issued Jun. 10, 1952. Aminopolyphosphonate compounds are excellent water conditioning agents and may be advantageously used in the present invention. Suitable examples include soluble salts, e.g. sodium, lithium or potassium salts, of diethylene thiamine pentamethylene phosphonic acid, ethylene diamine tetramethylene phosphonic acid, hexamethylenediamine tetramethylene phosphonic acid, and nitrilotrimethylene phosphonic acid; and, mixtures thereof. Water soluble short chain carboxylic acid salts constitute another class of water conditioner for use herein. Examples include citric acid, gluconic acid and phytic acid. Preferred salts are prepared from alkali metal ions such as sodium, potassium, lithium and from ammonium and substituted ammonium. Suitable water soluble polycarboxylate water conditioners for this invention include the various ether polycarboxylates, polyacetal, polycarboxylates, epoxy polycarboxylates, and aliphatic-, cycloalkane- and aromatic polycarboxylates.The composition may comprise:

a. From 0.008 wt% enzyme protein to 0.16 wt% enzyme protein of an enzyme having proteolytic activity, which enzyme is selected from the group consisting of:
    i. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus licheniformis*,
    ii. an enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1; and
b. from 0.5 wt. % to 1 wt. % of a surfactant;

c. from 0.5 wt% to 1 wt% of a carbonate source; and

d. water.

In one embodiment of the invention, the composition comprises

a. From 0.008 wt% enzyme protein to 0.16 wt% enzyme protein of an enzyme having proteolytic activity, which enzyme is selected from the group consisting of:

i. An enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1; and

b. from 0.5 wt. % to 1 wt. % of a surfactant;

c. from 0.5 wt% to 1 wt% of a carbonate source; and

d. water.

In one embodiment of the invention, the enzyme composition is a liquid composition.

In order to stabilize the enzymes in a liquid detergent composition, the composition can further comprise a protease inhibitor. The protease inhibitor inhibits the action of the enzyme having proteolytic activity and thus leads to a more shelf-stable detergent composition. In one embodiment of the invention the protease inhibitor is 4-formyl-phenyl-boronic acid.

In one embodiment of the invention, the protease inhibitor is a peptide aldehyde of the formula P-$(A)_y$-L-$(B)_x$-B^0-H or a hydrosulfite adduct or hemiacetal adduct thereof, wherein:

i. H is hydrogen;

ii. B^0 is a single amino acid residue with L- or D-configuration of the formula –NH-CH(R)-C(=O)-;

iii. x is 1, 2 or 3 for $(B)_x$, and B is independently a single amino acid connected to B^0 via the *C*-terminal of the $(B)_x$ amino acid

iv. L is absent or L is independently a linker group of the formula -C(=O)-, -C(=O)-C(=O)-, -C(=S)-, -C(=S)-C(=S)- or -C(=S)-C(=O)-;

v. y is 0, 1 or 2 for $(A)_y$, and A is independently a single amino acid residue connected to L via the *N*-terminal of the $(A)_y$ amino acid, with the proviso that if L is absent then A is absent;

vi. P is selected from the group consisting of hydrogen and an *N*-terminal protection group, with the proviso that if L is absent then P is an *N*-terminal protection group;

vii. R is independently selected from the group consisting of C_{1-6} alkyl, C_{6-10} aryl or C_{7-10} arylalkyl optionally substituted with one or more, identical or different, substituent's R';

viii. R' is independently selected from the group consisting of halogen, -OH, -OR'', -SH, -SR'', $-NH_2$, -NHR'', $-NR''_2$, $-CO_2H$, $-CONH_2$, -CONHR'', $-CONR''_2$, $-NHC(=N)NH_2$; and

ix. R'' is a C_{1-6} alkyl group.

In one embodiment, the hydrosulfite adduct of a peptide aldehyde is of the formula P-$(A)_y$-L-$(B)_x$-N(H)-CHR-CH(OH)-SO_3M, wherein

i. M is hydrogen or an alkali metal;

ii. x is 1, 2 or 3 for $(B)_x$, and B is independently a single amino acid connected to B^0 via the *C*-terminal of the $(B)_x$ amino acid

iii. L is absent or L is independently a linker group of the formula -C(=O)-, -C(=O)-C(=O)-, -C(=S)-, -C(=S)-C(=S)- or -C(=S)-C(=O)-;

iv. y is 0, 1 or 2 for $(A)_y$, and A is independently a single amino acid residue connected to L via the *N*-terminal of the $(A)_y$ amino acid, with the proviso that if L is absent then A is absent;

v. P is selected from the group consisting of hydrogen and an *N*-terminal protection group, with the proviso that if L is absent then P is an *N*-terminal protection group;

vi. R is independently selected from the group consisting of C_{1-6} alkyl, C_{6-10} aryl or C_{7-10} arylalkyl optionally substituted with one or more, identical or different, substituent's R';

vii. R' is independently selected from the group consisting of halogen, -OH, -OR'', -SH, -SR'', $-NH_2$, -NHR'', $-NR''_2$, $-CO_2H$, $-CONH_2$, -CONHR'', $-CONR''_2$, $-NHC(=N)NH_2$; and

viii. R'' is a C_{1-6} alkyl group.

In one embodiment, M is Na or K and R is a C7 arylalkyl substituted with –OH.

In one embodiment, B0 is selected from the group consisting of D- or L-form of arginine (Arg), 3,4-dihydroxyphenylalanine, isoleucine (Ile), leucine (Leu), methionine (Met), norleucine (Nle), norvaline (Nva), phenylalanine (Phe), m-tyrosine, p-tyrosine (Tyr) and valine (Val).

In one embodiment, B1 can be selected from the group consisting of alanine (Ala), cysteine (Cys), glycine (Gly), isoleucine (Ile), leucine (Leu), norleucine (Nle), norvaline (Nva), proline (Pro), serine (Ser), threonine (Thr) and valine (Val).

In one embodiment, B2 can be selected from the group consisting of alanine (Ala), arginine (Arg), capreomycidine (Cpd), cysteine (Cys), glycine (Gly), isoleucine (Ile), leucine (Leu), norleucine (Nle), norvaline (Nva), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), and valine (Val).

In one embodiment, B3 can be selected from the group consisting of isoleucine (Ile), leucine (Leu), norleucine (Nle), norvaline (Nva), phenylalanine (Phe), phenylglycine, tyrosine (Tyr), tryptophan (Trp) and valine (Val).

x can be 1, 2 or 3.

In one embodiment, A1 can be selected from the group consisting of alanine (Ala), arginine (Arg), capreomycidine (Cpd), glycine (Gly), isoleucine (Ile), leucine (Leu), norleucine (Nle), norvaline (Nva), phenylalanine (Phe), threonine (Thr), tyrosine (Tyr), tryptophan (Trp) and valine (Val).

In one embodiment, A2 can be selected from the group consisting of arginine (Arg), isoleucine (Ile), leucine (Leu), norleucine (Nle), norvaline (Nva), phenylalanine (Phe), phenylglycine, Tyrosine (Tyr), tryptophan (Trp) and valine (Val).

L can be absent and A is absent.

In one embodiment, P is selected from the group consisting of formyl, acetyl (Ac), benzoyl (Bz), trifluoroacetyl, methoxysuccinyl, fluorenylmethyloxycarbonyl (Fmoc), methoxycarbonyl (MEO-CO), (fluoromethoxy)carbonyl, benzyloxycarbonyl (Cbz), t-butyloxycarbonyl (Boc), adamantyloxycarbonyl, p-methoxybenzyl carbonyl (Moz), benzyl (Bn), p-methoxybenzyl (PMB), p-methoxyphenyl (PMP), methoxyacetyl, methylamino carbonyl (MeNCO), methylsulfonyl (MeSO2), ethylsulfonyl (EtSO2), benzylsulfonyl (PhCH2SO2), methylphosphoramidyl (MeOP(OH)(=O)) and benzylphosphoramidyl (PhCH2O-P(OH)(O)).

In one embodiment, the peptide aldehyde adduct is L-Alaninamide, N-[(phenylmethoxy)carbonyl]glycyl-N-[2-hydroxy-1-[(4-hydroxyphenyl)methyl]-2-sulfoethyl]-, sodium salt (1:1).

In one embodiment, the detergent composition comprises a protease inhibitor, which protease inhibitor is one of the following peptide aldehydes or a adduct thereof: Cbz-Arg-Ala-Tyr-H, Ac-Gly-Ala-Tyr-H, Cbz-Gly-Ala-Tyr-H, Cbz-Gly-Ala-Leu-H, Cbz-Val-Ala-Leu-H, Cbz-Gly-Ala-Phe-H, Cbz-Gly-Ala-Val-H, Cbz-Gly-Gly-Tyr-H, Cbz-Gly-Gly-Phe-H, Cbz-Arg-Val-Tyr-H, Cbz-Leu-Val-Tyr-H, Ac-Leu-Gly-Ala-Tyr-H, Ac-Phe-Gly-Ala-Tyr-H, Ac-Tyr-Gly-Ala-Tyr-H, Ac-Phe-Gly-Ala-Leu-H, Ac-Phe-Gly-Ala-Phe-H, Ac-Phe-Gly-Val-Tyr-H, Ac-Phe-Gly-Ala-Met-H, Ac-Trp-Leu-Val-Tyr-H, MeO-CO-Val-Ala-Leu-H, MeNCO-Val-Ala-Leu-H, MeO-CO-Phe-Gly-Ala-Leu-H, MeO-CO-Phe-Gly-Ala-Phe-H, MeSO2-Phe-Gly-Ala-Leu-H, MeSO2-Val-Ala-Leu-H, PhCH2O-P(OH)(O)-Val-Ala-Leu-H, EtSO2-Phe-Gly-Ala-Leu-H, PhCH2SO2-Val-Ala-Leu-H, PhCH2O-P(OH)(O)-Leu-Ala-Leu-H, PhCH2O-P(OH)(O)-Phe-Ala-Leu-H, or MeO-P(OH)(O)-Leu-Gly-Ala-Leu-H, α-MAPI, β-MAPI, Phe-C(=O)-Arg-Val-Tyr-H, Phe-C(=O)-Gly-Gly-Tyr-H, Phe-C(=O)-Gly-Ala-Phe-H, Phe-C(=O)-Gly-Ala-Tyr-H, Phe-C(=O)-Gly-Ala-L-H, Phe-C(=O)-Gly-Ala-Nva-H, Phe-C(=O)-Gly-Ala-Nle-H, Tyr-C(=O)-Arg-Val-Tyr-H, Tyr-C(=O)-Gly-Ala-Tyr-H, Phe-C(=S)-Arg-Val-Phe-H, Phe-C(=S)-Arg-Val-Tyr-H, Phe-C(=S)-Gly-Ala-Tyr-H, Antipain, GE20372A, GE20372B, Chymostatin A, Chymostatin B, Chymostatin C, Cbz-Gly-Ala-N(H)-

CH(CH2-p-C6H4OH)-CH(OH)-SO3Na, Cbz-Gly-Ala-N(H)-CH(CH2Ph)-CH(OH)-SO3Na, MeO-CO_Val-Ala-N(H)-CH(CH2CH(CH3)2)-CH(OH)-SO3Na,
Cbz-Arg-Ala-NHCH(CH2C6H4OH)C(OH)(SO3M)-H where M=Na,
Ac-Gly-Ala-NHCH(CH2C6H4OH)C(OH)(SO3M)-H, where M=Na,
Cbz-Gly-Ala-NHCH(CH2C6H4OH)C(OH)(SO3M)-H, where M=Na,
Cbz-Gly-Ala-NHCH(CH2CH(CH3)2))C(OH)(SO3M)-H, where M=Na,
Cbz-Val-Ala-NHCH(CH2CH(CH3)2))C(OH)(SO3M)-H, where M=Na,
Cbz-Gly-Ala-NHCH(CH2Ph)C(OH)(SO3M)-H, where M=Na,
Cbz-Gly-Ala-NHCH(CH(CH3)2)C(OH)(SO3M)-H, where M=Na,
Cbz-Gly-Gly-NHCH(CH2C6H4OH)C(OH)(SO3M)-H, where M=Na,
Cbz-Gly-Gly-NHCH(CH2Ph)C(OH)(SO3M)-H, where M=Na,
Cbz-Arg-Val-NHCH(CH2C6H4OH)C(OH)(SO3M)-H, where M=Na,
Cbz-Leu-Val-NHCH(CH2C6H4OH)C(OH)(SO3M)-H, where M=Na,
Ac-Leu-Gly-Ala-NHCH(CH2C6H4OH)C(OH)(SO3M)-H, where M=Na,
Ac-Phe-Gly-Ala-NHCH(CH2C6H4OH)C(OH)(SO3M)-H, where M=Na,
Ac-Tyr-Gly-Ala-NHCH(CH2C6H4OH)C(OH)(SO3M)-H, where M=Na,
Ac-Phe-Gly-Ala-NHCH(CH2CH(CH3)2))C(OH)(SO3M)-H, where M=Na,
Ac-Phe-Gly-Ala-NHCH(CH2Ph)C(OH)(SO3M)-H, where M=Na,
Ac-Phe-Gly-Val-NHCH(CH2C6H4OH)C(OH)(SO3M)-H, where M=Na ,
Ac-Phe-Gly-Ala-NHCH(CH2CH2SCH3)(SO3M)-H, where M=Na,
Ac-Trp-Leu-Val-NHCH(CH2C6H4OH)C(OH)(SO3M)-H, where M=Na,
MeO-CO-Val-Ala-NHCH(CH2CH(CH3)2))C(OH)(SO3M)-H, where M=Na,
MeNCO-Val-Ala-NHCH(CH2CH(CH3)2))C(OH)(SO3M)-H, where M=Na,
MeO-CO-Phe-Gly-Ala-NHCH(CH2CH(CH3)2))C(OH)(SO3M)-H, where M=Na,
MeO-CO-Phe-Gly-Ala-NHCH(CH2Ph)C(OH)(SO3M)-H, where M=Na,
MeSO2-Phe-Gly-Ala-NHCH(CH2CH(CH3)2))C(OH)(SO3M)-H, where M=Na,
MeSO2-Val-Ala-NHCH(CH2CH(CH3)2))C(OH)(SO3M)-H, where M=Na,
PhCH2O(OH)(O)P-Val-Ala-NHCH(CH2CH(CH3)2))C(OH)(SO3M)-H, where M=Na ,
EtSO2-Phe-Gly-Ala-NHCH(CH2CH(CH3)2))C(OH)(SO3M)-H, where M=Na,
PhCH2SO2-Val-Ala-NHCH(CH2CH(CH3)2))C(OH)(SO3M)-H, where M=Na,
PhCH2O(OH)(O)P-Leu-Ala-NHCH(CH2CH(CH3)2))C(OH)(SO3M)-H, where M=Na,
PhCH2O(OH)(O)P-Phe-Ala-NHCH(CH2CH(CH3)2))C(OH)(SO3M)-H, where M=Na,
MeO(OH)(O)P-Leu-Gly-Ala-NHCH(CH2CH(CH3)2))C(OH)(SO3M)-H, where M=Na, and
Phe-urea-Arg-Val-NHCH(CH2C6H4OH)C(OH)(SO3M)-H where M=Na.

The invention further relates to a method for cleaning-in-place wherein a wash liquor is circulated in a production or a process equipment and wherein the wash liquor comprises a carbonate source and an enzyme selected from the group consisting of:

a. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus licheniformis*,
b. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus halodurans*,
c. an enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1; and
d. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3.

The method provides a good cleaning performance on fermentation soil, which is comparable to benchmark cleaning and at the same time allows use of mild alkaline detergents. As no harsh chemical are used so the occupational health and safety for the employees carrying out the CIP is improved. The method allows use of low temperature in the wash liquor (10-30°C) and still the cleaning performance is comparable to benchmark cleaning. The cleaning performance of the method is comparable to benchmark cleaning eventhough the time for cleaning is considerably shorter because it is not necessary to vent the equipment and because the specific enzymes used in the method clean effectively. Another advantage is extension of the equipment lifetime due to application of inventive cleaning composition which is non-corrosive.

Furthermore, the specific enzymes used in the method ensure that the wash liquor does not foam as much as when other enzymes with same activity are used. This is due to the low foaming property of the specific enzymes having proteolytic activity.

According to the invention, the method comprises cleaning-in-place of production or process equipment, which method comprises the following steps:

a. Optionally pre-rinse by circulating water in the equipment and subsequently drain water;
b. Circulate in the equipment a wash liquor (i) or a wash liquor (ii) for a sufficient period of time:
    i. Wash liquor comprising the detergent composition of the present invention; or
    ii. Wash liquor comprising the enzyme having proteolytic activity,
c. Optionally stop circulation under step b and allow the process or production equipment to soak in the wash liquor;
d. Drain the wash liquor;

e. Rinse by circulating water in the equipment and subsequently drain water;

wherein steps (a), (b), (c), (d) or (e) are carried out one, two or three times.

The present invention method has the advantage the the drained wash liquor in step (d) (waste water) is less polluted than wash liquor from a benchmark cleaning, because harsh chemicals are not used in the present method.

The method may comprise an enzyme having proteolytic activity where the enzyme used in the method is derived from *Bacillus licheniformis*. The method may comprise an enzyme having proteolytic activity, where the enzyme used in the method is derived from *Bacillus halodurans*. The composition may comprise an enzyme having proteolytic activity, where the enzyme used in the method has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1. The composition may comprise an enzyme having proteolytic activity where the enzyme used in the method has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3.

The cleaning-in-place can be further improved by using one or more enzyme in combination with the enzyme composition. For example a further enzyme can be used in addition to the enzyme composition under step b defined above. Examples of the enzymes that can be used are one or more enzymes selected from: hemicellulases, peroxidases, proteases, cellulases, xylanases, lipases, phospholipases, esterases, cutinases, pectinases, mannanases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, pullulanases, tannases, pentosanases, malanases, ß-glucanases, arabinosidases, hyaluronidase, chondroitinase, laccase, chlorophyllases, amylases, perhydrolases, peroxidases or mixtures thereof.

In one embodiment, the method comprises use of an enzyme having amylolytic activity. In one embodiment the composition comprises comprises an enzyme having cellulolytic activity.

The method may comprise use of an enzyme having amylolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and

b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

The method may comprise use of an enzyme having cellulolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;
b. an enzyme derived from *Humicola insolens*; and
c. an enzyme derived from *Trichoderma reesei*.

In one embodiment of the invention, the method may comprise use of an enzyme having proteolytic activity derived from *Bacillus licheniformis* together with an enzyme having amylolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and
b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

In one embodiment, the method may comprise use of an enzyme having proteolytic activity derived from *Bacillus licheniformis* together with an enzyme having cellulolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;
b. an enzyme derived from *Humicola insolens*; and
c. an enzyme derived from *Trichoderma reesei*.

In one embodiment, the method may comprise use of an enzyme having proteolytic activity derived from *Bacillus halodurans* together with an enzyme having amylolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and
b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

In one embodiment, the method may comprise use of an enzyme having proteolytic activity derived from *Bacillus halodurans* together with an enzyme having cellulolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;
b. an enzyme derived from *Humicola insolens*; and
c. an enzyme derived from *Trichoderma reesei*.

In one embodiment, the method may comprise use of an enzyme having proteolytic activity having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1 and an enzyme having amylolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and
b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

In one embodiment, the method may comprise use of an enzyme having proteolytic activity haivng at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and an enzyme having cellulolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;
b. an enzyme derived from *Humicola insolens*; and
c. an enzyme derived from *Trichoderma reesei*.

In one embodiment, the method may comprise use of an enzyme having proteolytic activity and having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100%

sequence identity to amino acids 1-268 of SEQ ID NO: 3 and an enzyme having amylolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and
b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

In one embodiment, the method may comprise use of an enzyme having proteolytic activity and having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3, and an enzyme having cellulolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;
b. an enzyme derived from *Humicola insolens*; and
c. an enzyme derived from *Trichoderma reesei*.

In one embodiment, the method may comprise use of an enzyme having proteolytic activity having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1 and an enzyme having amylolytic activity having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and an enzyme having cellulolytic activity having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;

In one embodiment, the method may comprise use of an enzyme having proteolytic activity having 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and an enzyme having amylolytic activity having 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and an enzyme having cellulolytic activity having 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;

In one embodiment, the method may further comprise the use of at least one surfactant, at least one surfactant system, at least one soap, or any mixtures thereof. The kind of surfactant and/or soap is outlined above.

By using the method of the invention the cleaning performance is comparable to the traditional cleaning with NaOH (benchmark cleaning) and cleaning with harsh chemicals can thereby be omitted. The cleaning performance measured with Assay I is comparable to benchmark cleaning. The cleaning performance measured with Assay II is comparable to benchmark cleaning.

The method may comprise use of a protease inhibitor in the wash liquor. The protease inhibitor prolongs the action of the enzymes in the wash liquor as the inhibitor prevents degradation of the enzymes by the enzymes having proteolytic activity.

The wash liquor may be in the range of 10-30°C, such as in the range of 10-25°C, in the range of 15-25°C, in the range of 15-20°C or in the range of 18-22°C.

The pH of the wash liquor may be in the range of 6-11, such as pH of the wash liquor is 6, 7, 8, 9, 10 or 11.

In one embodiment of the invention, the time period for circulating the wash liquor in the equipment under step (b) is in the range of 20 to 90 minutes, such as in the range of 20 minutes to 80 minutes, in the range of 20 minutes to 70 minutes, in the range of 20 minutes to 60 minutes, in the range of 25 minutes to 80 minutes, in the range of 25 minutes to 70 minutes, in the range of 25 minutes to 60 minutes, in the range of 30 minutes to 80 minutes, in the range of 30 minutes to 70 minutes or in the range of 30 minutes to 60 minutes.

The method further comprises dismantling of the production or process equipment and soaking the equipment in the wash liquor. This would allow very dirty parts of the equipment to undergo intense cleaning.

In one embodiment of the invention, the process or production equipment is used in food industry, pharma industry and biotech industry can be cleaned with the enzyme having proteolytic activity. Especially, process or production equipment used in the brewing industry, dairy industry, pharma industry in biotech industry.

In one embodiment of the invention, the enzyme is especially good for cleaning process or production equipment for brewing, such as fermentation tanks for fermentation of the beer or wine.

Traditional CIP is quite time consuming, requires harch chemicals during the process. A traditional CIP process uses harch chemicals like strong acids and strong bases and takes about 1½-2 hours. Thus with the inventive method is more environmental friendly and time and energy is saved. In short, the inventive method is therefore more friendly to the environment and less costly. The method at the same time ensures improved cleaning performance.

The method may further comprise in-activating enzymes remaining in the production equipment after steps (a) to (e) have been carried out. The enzymes can be in-activated by circulating an acidic solution in the equipment, rinsing the equipment by circulating water in the equipment and subsequently drain water.

The acidic solution used for in-acitivating the enzymes have a pH in the range of 1 to 3, such as in the range of 1.5 to 2. The acidic solution may comprise nitric acid and citric acid. This combination of acids are better for the environment that using strong acids.

The present method for cleaning-in-place can be used for reducing and/or removing fermentation soil in equipment used in food industry, pharma industry and biotech industry. However, the method is particular useful for equipment used for food and beverage manufacturing.

In order to further enhance the cleaning of specific equipment, the equipment may be dismantled and soaked in the wash liquor. For example filters or membranes, such as semipermeable membranes used for e.g. ultrafiltration, are difficult. The membranes can soaked in the wash liquor during the cleaning-in-place or if dismantled for further cleaning.

The concentration of an enzyme having proteolytic activity in the wash liquor is in the range of 20-1540 mg enzyme protein/liter, such as in the range of 20-1400 mg enzyme protein/liter, in the range of 30-1300 mg enzyme protein/liter, in the range of 35-1200 mg enzyme protein/liter, in the range of 40-1100 mg enzyme protein/liter, in the range of 45-1000 mg enzyme protein/liter, in the range of 50-900 mg enzyme protein/liter, in the range of 55-800 mg enzyme protein/liter, in the range of 60-700 mg enzyme protein/liter, in the range of 60-600 mg enzyme protein/liter, in the range of 60-500 mg enzyme protein/liter, in the range of 60-400 mg enzyme protein/liter, in the range of 60-300 mg enzyme protein/liter, in the range of 60-200 mg enzyme protein/liter, in the range of 60-100 mg enzyme protein/liter, in the range of 65-90 mg enzyme protein/liter or in the range of 70-80 mg enzyme protein/liter.

Or the concentration of an enzyme having amylase activity in the wash liquor is in the range of 5-360 mg enzyme protein/liter, such as in the range of 6-300 mg enzyme protein/liter, in the range of 7-200 mg enzyme protein/liter, in the range of 8-100 mg enzyme protein/liter, in the range of 9-50 mg enzyme protein/liter, in the range of 10-40 mg enzyme protein/liter, in the range of 11-30 mg enzyme protein/liter, in the range of 12-25 mg enzyme protein/liter, in the range of 13-20 mg enzyme protein/liter, in the range of 15-20 mg enzyme protein/liter,

The concentration of an enzyme having cellulolytic activity in the wash liquor is in the range of 1-40 mg enzyme protein/liter, such as in the range of 1-25 mg enzyme protein/liter, in the range of 1-20 mg enzyme protein/liter, in the range of 1-15 mg enzyme protein/liter, in the range of 1-10 mg enzyme protein/liter, in the range of 1-5 mg enzyme protein/liter, in the range of 1.5-3 mg enzyme protein/liter or in the range of 1.5-2.5 mg enzyme protein/liter.

The invention is further defined in the following paragraphs:

1. Use of an enzyme for cleaning-in-place of process or production equipment, wherein the enzyme is selected from the group consisting of:
    a. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus licheniformis;*
    b. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus halodurans*;
    c. an enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1; and
    d. an enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3.

2. Use of an enzyme according to paragraph 1, wherein the process or production equipment is process or production equipment used in food industry, pharma industry and biotech industry.

3. Use of an enzyme according to any of paragraphs 1 or 2, wherein the process or production equipment is selected from the group consisting of process or production equipment used in brewing industry, process or production equipment used in dairy industry, process or production equipment used in pharma industry and process or production equipment used in biotech industry.

4. Use of an enzyme according to any of paragraphs 1-3, wherein the process or production equipment is process or production equipment for brewing.

5. Use of an enzyme according to any of the preceding paragraphs, wherein the process or production equipment is selected from the group consisting of road tankers, processing tanks, storage tanks, fermentation tanks, pipelines, heat-exchangers, homogenizers, filter units, filter membranes and packaging lines.

6. Use of an enzyme according to any of the preceding paragraphs, wherein the production equipment is a fermentation tank.

7. Use of an enzyme according to any of the preceding paragraphs, wherein the enzyme reduces or removes the fermentation soil from the process or production equipment.

8. Use of an enzyme according to any of the preceding paragraphs, wherein the enzyme has low foaming properties.

9. Use of an enzyme according to any of the preceding paragraphs, wherein the enzyme is derived from *Bacillus licheniformis*.

10. Use of an enzyme according to any of paragraphs 1-8, wherein the enzyme is derived from *Bacillus halodurans*.

11. Use of an enzyme according to any of paragraphs 1-9, wherein the enzyme has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1.

12. Use of an enzyme according to any of paragraphs 1-8, wherein the enzyme has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3.

13. Use of an enzyme according to any of the preceding paragraphs, wherein the use further comprises using one or more enzymes selected from: hemicellulases, peroxidases, proteases, cellulases, xylanases, lipases, phospholipases, esterases, cutinases, pectinases, mannanases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, pullulanases, tannases, pentosanases, malanases, ß-glucanases, arabinosidases, hyaluronidase, chondroitinase, laccase, chlorophyllases, amylases, perhydrolases, peroxidases or mixtures thereof.

14. Use according to paragraph 13, wherein the use further comprises using an enzyme having amylolytic activity.

15. Use according to any of paragraphs 13-14, wherein the use further comprises using an enzyme having cellulolytic activity.

16. Use according to any of paragraphs 13-15, wherein the enzyme having amylolytic activity is selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and

b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

17. Use according to any of paragraphs 13-16, wherein the enzyme having cellulolytic activity is selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;

b. an enzyme derived from *Humicola insolens;* and

c. an enzyme derived from *Trichoderma reesei*.

18. Use according to any of paragraphs 1-9 and 13-17, wherein the enzyme having proteolytic activity is derived from *Bacillus licheniformis*, and is used together with an enzyme having amylolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and

b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

19. Use according to any of paragraphs 1-9 and 13-17, wherein the enzyme having proteolytic activity is derived from *Bacillus licheniformis*, and is used together with an enzyme having cellulolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;

b. an enzyme derived from *Humicola insolens;* and
c. an enzyme derived from *Trichoderma reesei*.

20. Use according to any of paragraphs 1-8, 10 and 13-17, wherein the enzyme having proteolytic activity is derived from *Bacillus halodurans*, and is used together with an enzyme having amylolytic activity selected from the group consisting of:
    a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and
    b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

21. Use according to any of paragraphs 1-8, 10 and 13-17, wherein the enzyme having proteolytic activity is derived from *Bacillus halodurans*, and is used together with an enzyme having cellulolytic activity selected from the group consisting of:
    a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;
    b. an enzyme derived from *Humicola insolens*; and
    c. an enzyme derived from *Trichoderma reesei.*

22. Use according to any of paragraphs 1-8, 11 and 13-17, wherein the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having amylolytic activity selected from the group consisting of:
    a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and
    b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least

97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

23. Use according to any of paragraphs 1-8, 11 and 13-17, wherein the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having cellulolytic activity selected from the group consisting of:
    a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;
    b. an enzyme derived from *Humicola insolens*; and
    c. an enzyme derived from *Trichoderma reesei*.

24. Use according to any of paragraphs 1-8, 12 and 13-17, wherein the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3 and is used together with an enzyme having amylolytic activity selected from the group consisting of:
    a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and
    b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

25. Use according to any of paragraphs 1-8, 12 and 13-17, wherein the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3, and is used together with an enzyme having cellulolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;
b. an enzyme derived from *Humicola insolens*; and
c. an enzyme derived from *Trichoderma reesei*.

26. Use according to any of paragraphs 1-8, 11, 13-17 and 22-23, wherein the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having amylolytic activity having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and an enzyme having cellulolytic activity having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;

27. Use according to any of paragraphs 1-8, 11, 13-17, 22-23 and 26, wherein the enzyme having proteolytic activity has 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having amylolytic activity having 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and an enzyme having cellulolytic activity having 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6.

28. Use of an enzyme according to any of the preceding paragraphs, further comprising use of at least one surfactant, at least one surfactant system, at least one soap, or any mixtures thereof.

29. Use of an enzyme according to paragraph 28, wherein the surfactant or the surfactant system comprises one or more non-ionic surfactants or one ore more amphoteric surfactant or a mixture thereof.

30. Use of an enzyme according to paragraph 29, wherein the non-ionic surfactant is selected from the group consisting of glycerol derivatives, sorbitan, glucose, sucrose derivatives, fatty acid ethoxylates, fatty acid ethoxylates propoxylates, fatty alcohol ethoxylates, alkyl phenol ethoxylates, fatty alcohol ethoxylates propoxylates, fatty esters of

polyalcohol ethoxylates, end-blocked ethoxylates, polypropylene glycols and polyethylene glycols.

31. Use of an enzyme according to paragraph 29, wherein the amphoteric surfactant is selected from alkylimidazoline, alkylbetaines, alkylamidobetaines and protein derivatives.

32. Use of an enzyme according to any of the preceding paragraphs, wherein the cleaning performance on fermentation soil is comparable to benchmark cleaning.

33. Use of an enzyme according to paragraph 32, wherein the cleaning performance on fermentation soil is comparable to benchmark cleaning when measured with Assay I.

34. Use of an enzyme according to paragraph 32, wherein the cleaning performance on fermentation soil is comparable to benchmark cleaning when measured with Assay II.

35. Use of an enzyme according to any of the preceding paragraphs, wherein the enzyme is used at a temperature in the range of 10-30°C.

36. Use of an enzyme according to paragraph 35, wherein the temperature is in the range of 10-25°C, such as in the range of 15-25°C, in the range of 15-20°C or in the range of 18-22°C.

37. Detergent composition for cleaning-in-place which detergent composition comprises
    a. From 0.001 wt. % enzyme protein to 0.16 wt. % enzyme protein of an enzyme having proteolytic activity, which enzyme is selected from the group consisting of:
        i. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus licheniformis*,
        ii. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus halodurans*,
        iii. An enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1; and
        iv. An enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3.

b. Optionally from 0.01 wt. % to 2 wt. % of a surfactant;
c. from 0.5 wt% to 2 wt% of a carbonate source; and
d. water.

38. Composition according to paragraph 37, wherein the enzyme is derived from *Bacillus licheniformis*.

39. Composition according to paragraph 37, wherein the enzyme is derived from *Bacillus halodurans*.

40. Composition according to paragraph 37, wherein the enzyme has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1.

41. Composition according to paragraph 37, wherein the enzyme has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3.

42. Composition according to any of the preceding composition paragraphs, wherein the composition further comprises one or more enzymes selected from: hemicellulases, peroxidases, proteases, cellulases, xylanases, lipases, phospholipases, esterases, cutinases, pectinases, mannanases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, pullulanases, tannases, pentosanases, malanases, ß-glucanases, arabinosidases, hyaluronidase, chondroitinase, laccase, chlorophyllases, amylases, perhydrolases, peroxidases or mixtures thereof.

43. Composition according to any of the preceding composition paragraphs, wherein the composition further comprises an enzyme having amylolytic activity.

44. Composition according to any of the preceding composition paragraphs, wherein the composition further comprises an enzyme having cellulolytic activity.

45. Composition according to paragraph 43, wherein the enzyme having amylolytic activity is selected from the group consisting of:
a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least

97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and

b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5;

46. Composition according to paragraph 44, wherein the enzyme having cellulolytic activity is selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;

b. an enzyme derived from *Humicola insolens;* and

c. an enzyme derived from *Trichoderma reesei*.

47. Composition according to any of paragraphs 37-38 and 42-45, wherein the enzyme having proteolytic activity is derived from *Bacillus licheniformis*, and is used together with an enzyme having amylolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and

b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

48. Composition according to any of paragraphs 37-38 and 42-46, wherein the enzyme having proteolytic activity is derived from *Bacillus licheniformis*, and is used together with an enzyme having cellulolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;

b. an enzyme derived from *Humicola insolens*; and

c. an enzyme derived from *Trichoderma reesei*.

49. Composition according to any of paragraphs 37, 39 and 42-46, wherein the enzyme having proteolytic activity is derived from *Bacillus halodurans*, and is used together with an enzyme having amylolytic activity selected from the group consisting of:
    a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and
    b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

50. Composition according to any of paragraphs 37, 39 and 42-46, wherein the enzyme having proteolytic activity is derived from *Bacillus halodurans*, and is used together with an enzyme having cellulolytic activity selected from the group consisting of:
    a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;
    b. an enzyme derived from *Humicola insolens*; and
    c. an enzyme derived from *Trichoderma reesei*.

51. Composition according to any of paragraphs 37, 40 and 42-46, wherein the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having amylolytic activity selected from the group consisting of:
    a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and
    b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

52. Composition according to any of paragraphs 37, 40 and 42-46, wherein the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having cellulolytic activity selected from the group consisting of:
    a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;
    b. an enzyme derived from *Humicola insolens*; and
    c. an enzyme derived from *Trichoderma reesei*.

53. Composition according to any of paragraphs 37, 41 and 42-46, wherein the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3 and is used together with an enzyme having amylolytic activity selected from the group consisting of:
    a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and
    b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

54. Composition according to any of paragraphs 37, 41 and 42-46, wherein the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3, and is used together with an enzyme having cellulolytic activity selected from the group consisting of:
    a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least

97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;

b. an enzyme derived from *Humicola insolens*; and

c. an enzyme derived from *Trichoderma reesei*.

55. Composition according to any of paragraphs 37, 40, 42-46 and 51-52, wherein the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having amylolytic activity having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and an enzyme having cellulolytic activity having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6.

56. Composition according to any of paragraphs 37, 40, 42-46,51,52 and 55, wherein the enzyme having proteolytic activity has 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having amylolytic activity having 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and an enzyme having cellulolytic activity having 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6.

57. Composition according to any of the preceding composition paragraphs, wherein the carbonate source is an aquous solution of carbonic acid, carbonate and/or bicarbonate optionally generated in situ.

58. Composition according to paragraph 57, wherein the carbonic source is selected from the group consisting of sodium carbonate, potassium carbonate, bicarbonate, sesquicarbonate and mixtures thereof.

59. Composition according to any of the preceding composition paragraphs further comprising at least one surfactant, at least one surfactant system, at least one soap, or any mixtures thereof.

60. Composition according to paragraph 59, wherein the surfactant is one or more non-ionic surfactants or one ore more amphoteric surfactant or a mixture thereof.

61. Composition according to paragraph 60, wherein the non-ionic surfactant is selected from the group consisting of glycerol derivatives, sorbitan, glucose, sucrose derivatives, fatty acid ethoxylates, fatty acid ethoxylates propoxylates, fatty alcohol ethoxylates, alkyl phenol ethoxylates, fatty alcohol ethoxylates propoxylates, fatty esters of polyalcohol ethoxylates, end-blocked ethoxylates, polypropylene glycols and polyethylene glycols.

62. Composition according to paragraph 61, wherein the amphoteric surfactant is selected from alkylimidazoline, alkylbetaines, alkylamidobetaines and protein derivatives.

63. Composition according to any of the preceding composition paragraphs, which composition comprises
    a. From 0.008 wt% enzyme protein to 0.16 wt% enzyme protein of an enzyme having proteolytic activity, which enzyme is selected from the group consisting of:
        i. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus licheniformis*,
        ii. An enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1; and
    b. from 0.5 wt. % to 1 wt. % of a surfactant;
    c. from 0.5 wt% to 1 wt% of a carbonate source; and
    d. water.

64. Composition according to paragraph 63, which composition comprises
    a. From 0.008 wt% enzyme protein to 0.16 wt% enzyme protein of an enzyme having proteolytic activity, which enzyme is selected from the group consisting of:
        i. An enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1; and
    b. from 0.5 wt. % to 1 wt. % of a surfactant;
    c. from 0.5 wt% to 1 wt% of a carbonate source; and
    d. water.

65. Composition according to any of the preceding composition paragraphs, wherein the composition is a liquid composition.

66. Composition according to any of the preceding composition paragraphs, wherein the composition comprises a protease inhibitor.

67. Composition according to paragraph 66, wherein the protease inhibitor is 4-formyl-phenyl-boronic acid or a peptide a peptide aldehyde of the formula P-$(A)_y$-L-$(B)_x$-B^0-H or a hydrosulfite adduct or hemiacetal adduct thereof, wherein:

i. H is hydrogen;
ii. B^0 is a single amino acid residue with L- or D-configuration of the formula –NH-CH(R)-C(=O)-;
iii. x is 1, 2 or 3 for $(B)_x$, and B is independently a single amino acid connected to B^0 via the *C*-terminal of the $(B)_x$ amino acid
iv. L is absent or L is independently a linker group of the formula -C(=O)-, -C(=O)-C(=O)-, -C(=S)-, -C(=S)-C(=S)- or -C(=S)-C(=O)-;
v. y is 0, 1 or 2 for $(A)_y$, and A is independently a single amino acid residue connected to L via the *N*-terminal of the $(A)_y$ amino acid, with the proviso that if L is absent then A is absent;
vi. P is selected from the group consisting of hydrogen and an *N*-terminal protection group, with the proviso that if L is absent then P is an *N*-terminal protection group;
vii. R is independently selected from the group consisting of C_{1-6} alkyl, C_{6-10} aryl or C_{7-10} arylalkyl optionally substituted with one or more, identical or different, substituent's R';
viii. R' is independently selected from the group consisting of halogen, -OH, -OR'', -SH, -SR'', $-NH_2$, -NHR'', $-NR''_2$, $-CO_2H$, $-CONH_2$, -CONHR'', $-CONR''_2$, $-NHC(=N)NH_2$; and
ix. R'' is a C_{1-6} alkyl group.

68. Composition according to paragraph 67, wherein the peptide aldehyde adduct is L-Alaninamide, *N*-[(phenylmethoxy)carbonyl]glycyl-*N*-[2-hydroxy-1-[(4-hydroxyphenyl)methyl]-2-sulfoethyl]-, sodium salt (1:1).

69. A method for cleaning-in-place wherein a wash liquor is circulated in a production or a process equipment and wherein the wash liquor comprises a carbonate source and an enzyme selected from the group consisting of:

a. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus licheniformis*;
b. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus halodurans*;
c. an enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1; and
d. an enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3.

70. Method according to paragraph 69, wherein the method comprises cleaning-in-place of production or process equipment, which method comprises the following steps:
a. Optionally pre-rinse by circulating water in the equipment and subsequently drain water;
b. circulate in the equipment a wash liquor (i) or a wash liquor (ii) for a sufficient period of time:
i. wash liquor comprising the detergent composition of any of paragraphs 37-68; or
ii. wash liquor comprising the enzyme having proteolytic activity;
c. optionally stop circulation under step b and allow the process or production equipment to soak in the wash liquor;
d. drain the wash liquor;
e. rinse by circulating water in the equipment and subsequently drain water;
wherein steps a), (b), (c), (d) or (e) are carried out one, two or three times.

70. Method according to any of paragraphs 69-70, wherein the enzyme is derived from *Bacillus licheniformis*.

71. Method according to any of paragraphs 69-70, wherein the enzyme is derived from *Bacillus halodurans*.

72. Method according to any of paragraphs 69-70, wherein the enzyme has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity,

at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1.

73. Method according to any of paragraphs 69-70, wherein the enzyme has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3.

74. Method according to any of the preceding method paragraphs, wherein wash liquor further comprises one or more enzymes selected from: hemicellulases, peroxidases, proteases, cellulases, xylanases, lipases, phospholipases, esterases, cutinases, pectinases, mannanases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, pullulanases, tannases, pentosanases, malanases, ß-glucanases, arabinosidases, hyaluronidase, chondroitinase, laccase, chlorophyllases, amylases, perhydrolases, peroxidases or mixtures thereof.

75. Method according to any of the preceding method paragraphs, wherein the wash liquor further comprises an enzyme having amylolytic activity.

76. Method according to any of the preceding method paragraphs, wherein the wash liquor further comprises an enzyme having cellulolytic activity.

77. Method according to paragraph 75, wherein the enzyme having amylolytic activity is selected from the group consisting of:
    a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and
    b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

78. Method according to paragraph 76, wherein the enzyme having cellulolytic activity is selected from the group consisting of:
    a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least

97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;

b. an enzyme derived from *Humicola insolens*; and

c. an enzyme derived from *Trichoderma reesei*.

79. Method according to any of paragraphs 68-70 and 77, wherein the enzyme having proteolytic activity is derived from *Bacillus licheniformis*, and is used together with an enzyme having amylolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and

b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

80. Method according to any of paragraphs 68-70 and 78, wherein the enzyme having proteolytic activity is derived from *Bacillus licheniformis*, and is used together with an enzyme having cellulolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;

b. an enzyme derived from *Humicola insolens*; and

c. an enzyme derived from *Trichoderma reesei*.

81. Method according to any of paragraphs 68-69, 71 and 77, wherein the enzyme having proteolytic activity is derived from *Bacillus halodurans*, and is used together with an enzyme having amylolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and

b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least

97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

82. Method according to any of paragraphs 68-69, 71 and 78, wherein the enzyme having proteolytic activity is derived from *Bacillus halodurans*, and is used together with an enzyme having cellulolytic activity selected from the group consisting of:
    a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;
    b. an enzyme derived from *Humicola insolens*; and
    c. an enzyme derived from *Trichoderma reesei*.

83. Method according to any of paragraphs 68-69, 72 and 77, wherein the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having amylolytic activity selected from the group consisting of:
    a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and
    b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

84. Method according to any of paragraphs 68-69, 72 and 78, wherein the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having cellulolytic activity selected from the group consisting of:
    a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least

97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;

b. an enzyme derived from *Humicola insolens*; and

c. an enzyme derived from *Trichoderma reesei*.

85. Method according to any of paragraphs 68-69, 73 and 77, wherein the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3 and is used together with an enzyme having amylolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and

b. an enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 5.

86. Method according to any of paragraphs 68-69, 73 and 78, wherein the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3, and is used together with an enzyme having cellulolytic activity selected from the group consisting of:

a. An enzyme having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6;

b. an enzyme derived from *Humicola insolens*; and

c. an enzyme derived from *Trichoderma reesei*.

87. Method according to any of paragraphs 68-69, 72, 77-78 and 83-84, wherein the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having amylolytic activity having at least

60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and an enzyme having cellulolytic activity having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6.

88. Method according to any of paragraphs 68-69, 72, 74-78, 77-78, 83-84 and 87, wherein the enzyme having proteolytic activity has 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having amylolytic activity having 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and an enzyme having cellulolytic activity having 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6.

89. Method according to any of the preceding method paragraphs, further comprising use of at least one surfactant, at least one surfactant system, at least one soap, or any mixtures thereof.

90. Method according to any of the preceding method paragraphs, wherein the cleaning performance on fermentation soil is comparable to benchmark cleaning.

91. Method according to paragraph 90, wherein the cleaning performance on fermentation soil is comparable to benchmark cleaning when measured with Assay I.

92. Method according to paragraph 90, wherein the cleaning performance on fermentation soil is comparable to benchmark cleaning when measured with Assay II.

93. Method according to any of the preceding method paragraphs, wherein the method comprises use of a protease inhibitor in the wash liquor.

94. Method according to any of the preceding method paragraphs, wherein the temperature of the wash liquor is in the range of 10-30°C.

95. Method according to paragraph 94, wherein the temperature of the wash liquor is in the range of 10-25°C, such as in the range of 15-25°C, in the range of 15-20°C or in the range of 18-22°C.

96. Method according to any of the preceding method paragraphs, wherein the pH of the wash liquor is in the range of 7-11.

97. Method according to paragraph 96, wherein the pH of the wash liquor is 7, 8, 9, 10 or 11.

98. Method according to any of the preceding method paragraphs, wherein the time period for circulating the wash liquor in the equipment is in the range of 20 to 90 minutes, such as in the range of 20 minutes to 80 minutes, in the range of 20 minutes to 70 minutes, in the range of 20 minutes to 60 minutes, in the range of 25 minutes to 80 minutes, in the range of 25 minutes to 70 minutes, in the range of 25 minutes to 60 minutes, in the range of 30 minutes to 80 minutes, in the range of 30 minutes to 70 minutes or in the range of 30 minutes to 60 minutes.

99. Method according to any of the preceding method paragraphs, wherein the method further comprises dismantling of the production or process equipment and soaking the equipment in the wash liquor.

100. Method according to any of the preceding method paragraphs, wherein the process or production equipment is process or production equipment used in food industry, pharma industry and biotech industry.

101. Method according to any of the preceding method paragraphs, wherein the process or production equipment is selected from the group consisting of process or production equipment used in brewing industry, process or production equipment used in dairy industry, process or production equipment used in pharma industry and process or production equipment used in biotech industry.

102. Method according to any of the preceding method paragraphs, wherein the process or production equipment is process or production equipment for brewing.

103. Method according to any of the preceding method paragraphs, wherein the process or production equipment is selected from the group consisting of road tankers, processing tanks, storage tanks, fermentation tanks, pipelines, heat-exchangers, homogenizers, filter units, filter membranes and packaging lines.

104. Method according to any of the preceding method paragraphs, wherein the production equipment is a fermentation tank.

105. Method according to any of the preceding method paragraphs, wherein the method reduces or removes the fermentation soil from the process or production equipment.

106. Method according to any of the preceding method paragraphs, wherein the method further comprises in-activating enzymes in the production or process equipment by circulating an acidic solution in the equipment, rinsing by circulating water in the equipment and subsequently drain water.

107. Method according to paragraph 106, wherein the in-activation is carried out after step d or step e.

108. Mehod according to any of paragraphs 106-107, wherein the acidic solution has a pH in the range of 1 to 3, such as in the range of 1.5 to 2.

109. Method according to any of paragraphs 106-108, wherein the acidic solution comprises nitric acid and citric acid.

110. Method according to any of the preceding method paragraphs, wherein the concentration of the enzyme having proteolytic activity in the wash liquor is in the range of 20-1540 mg enzyme protein/liter, such as in the range of 20-1400 mg enzyme protein/liter, in the range of 30-1300 mg enzyme protein/liter, in the range of 35-1200 mg enzyme protein/liter, in the range of 40-1100 mg enzyme protein/liter, in the range of 45-1000 mg enzyme protein/liter, in the range of 50-900 mg enzyme protein/liter, in the range of 55-800 mg enzyme protein/liter, in the range of 60-700 mg enzyme protein/liter, in the range of 60-600 mg enzyme protein/liter, in the range of 60-500 mg enzyme protein/liter, in the range of 60-400 mg enzyme protein/liter, in the range of 60-300 mg enzyme protein/liter, in the range of 60-200 mg enzyme protein/liter, in the range of 60-100 mg enzyme protein/liter, in the range of 65-90 mg enzyme protein/liter or in the range of 70-80 mg enzyme protein/liter.

111. Method according to any of the preceding method paragraphs, wherein the concentration of the enzyme having amylase activity in the wash liquor is in the range of 5-360 mg enzyme protein/liter, such as in the range of 6-300 mg enzyme protein/liter, in the range of 7-200 mg enzyme protein/liter, in the range of 8-100 mg enzyme protein/liter, in the range of 9-50 mg enzyme protein/liter, in the range of 10-40 mg enzyme protein/liter, in the range of 11-30 mg enzyme protein/liter, in the range of 12-25

mg enzyme protein/liter, in the range of 13-20 mg enzyme protein/liter, in the range of 15-20 mg enzyme protein/liter.

112. Method according to any of the preceding method paragraphs, wherein the concentration of the enzyme having cellulolytic activity in the wash liquor is in the range of 1-40 mg enzyme protein/liter, such as in the range of 1-25 mg enzyme protein/liter, in the range of 1-20 mg enzyme protein/liter, in the range of 1-15 mg enzyme protein/liter, in the range of 1-10 mg enzyme protein/liter, in the range of 1-5 mg enzyme protein/liter, in the range of 1.5-3 mg enzyme protein/liter or in the range of 1.5-2.5 mg enzyme protein/liter.

113. Method according to paragraph 70, wherein the water in step d) includes a disinfecting agent.

Assays

Assay I – Determination of cleaning performance using mini wash assay

In order to determine the cleaning performance of enzymes on fermentation soil, mini wash experiments are performed using a mini wash robot. In this mini wash robot soiled stainless steel (DIN 1.4301/SAE grade 304) discs with a diameter of 40 mm can be washed in various wash liquors at the same time under the exact same conditions (time, temperature, and mechanical stress). The mini wash robot has a number of slots for wash liquor and holders for the stainless steel discs soiled as described below. These holders continuously lift the stainless steel discs into the wash liquor at a regular speed while at the same time allowing for constant contact between the wash liquor and the stainless steel discs. By end of a cleaning trial the stainless steel discs are rinsed with running deionized water. The washed and rinsed stainless steel discs are subsequently dried at 65°C overnight where after the cleaning performance is determined gravimetrically.

Gravimetric determination of cleaning performance is performed by weight determination of the removed soil in proportion to the initial amount of soil. The results are furthermore normalized, so that the wash performance of the benchmark, 1% NaOH, is set to 100.

Soiling of stainless steel discs:

For soiling of stainless steel discs for the mini wash assay, a fermentation is performed by the following recipe:

Weyermann Bavarian Pilsner	Water (ml)	Sugar (g)	Size reduced hop pellets

Malt Extract - Unhopped (g)			(g)
250	2250	100	2

Malt extract and sugar is dissolved in cold tap water and heated to boiling point. The size reduced hop pellets are added and the solution is left to boil for 1 hour. After boiling, the malt solution is placed in ice bath and cooled to 19-20°C before addition of yeast and aerated for 30 minutes using an aquarium pump (Sera precision air 550 R plus) delivering 9.2 liters of air/minute. The mixture is left to ferment for 24 hours at 21°C in a 10L fermentation plastic bucket.

After the fermentation process, the produced foam is harvested and made in to homogenous slurry consisting of yeast with foam on the top. The foam is distributed evenly on 48 stainless steel discs and 300microL of yeast slurry is added to each of the stainless steel discs. The discs are finally dried at 65°C over night and the weight of the amount of soil applied is determined before the mini wash experiments are performed.

Assay II – Determination of cleaning performance of soiled beer fermentation vessels by visual inspection

Fermentation vessels (fermentation tank), each with a volume of 10 HL and equipped with a 1/8" HWS rotating tank cleaning spray ball in the top (supplied by BETE), is soiled by fermentation of hopped 100% barley wort with 11.5 degree plato. Pitching is done with a bottom fermenting yeast (cell count of 10-15 million cells/ml) and the wort is left to ferment at 20°C until the final attenuation degree of 80% is reached. By end fermentation the green beer is cooled to -1°C for at least 24 hours before the fermentation vessels are emptied and subsequently rinsed with tap water by three flushes each for five seconds with 5 seconds pause in between each flush. Cleaning of the fermentation vessels is performed by recirculation of the wash liquor through the spray ball for 60 minutes. The cleaning is performed at a temperature between about 16°C and about 22°C. The pH of the wash liquor is measured using a handheld pH-meter before and after the cleaning cycle in order to evaluate a possible change in pH of the wash liquor. After 60 minutes of cleaning the fermentation vessels are rinsed with tap water by five flushes each for five seconds with 5 seconds pause in between each flush.

Cleaning performance is finally determined visually. Visual determination of the cleaning performance is performed by a trained test person who visually rates the degree of cleanliness of the production equipment on a scale from 1-5, where 1 is clean, 2 is lightly soiled, 3 is soiled, 4 is very soiled and 5 is heavily soiled.

Assay III

The foaming properties of enzymes and/or chemicals when in contact with fermentation

soil are investigated by use of a milk frother (available at Tiger store). Firstly, a fermentation is prepared as described in Assay I under soiling of stainless steel discs. After 24 hours of fermentation the foam is harvested and made into a homogenous slurry. 4 ml of slurry is distributed evenly in the bottom of a tall 250 ml glass beaker, where after the soiled beakers are dried at 65°C overnight. After drying, 50 ml of a wash liquor is added to the soiled beaker. The milk frother is applied for 2 minutes and the height (cm) of the generated foam is measured.

EXAMPLES

Example 1

The cleaning performances of two types of enzyme mixes in 50 mM carbonate buffer, pH 10 were evaluated using the mini wash assay (Assay I) described above.

The experiments were performed using 1% (wt.) NaOH as benchmark and 50 mM carbonate buffer, pH 10 and deionized water as control. A 1% (wt.) H_3PO_4 was included in the experiments for comparison. The experiments were furthermore conducted at room temperature (app. 20°C).

The experimental conditions for the wash trials are specified in the two tables below.

Test conditions for CIP using Assay I with a wash liquor comprising enzyme mix 1: SEQ ID NO: 1, SEQ ID NO: 4 and SEQ ID NO: 6 in 50 mM carbonate buffer, pH 10.

Buffer	50 mM Carbonate buffer, pH 10	
Benchmark	1% NaOH	
Controls	Deionized water and 50 mM carbonate buffer, pH 10	
Enzymes	Enzyme	
Enzyme dosage	SEQ ID NO: 1	15.4 mg enzyme protein (EP)/200 ml wash liquor
	SEQ ID NO: 4	3.6 mg EP/200 ml wash liquor
	SEQ ID NO: 6	0.4 mg EP/200 ml wash liquor
	Total enzyme dosage	19.4 mg EP/200 ml wash liquor
Wash liquor volume	200 ml	
Temperature of wash liquor	Room temperature	
Cleaning time	8 minutes	
Rinse time	15 seconds	
Test system,	Soiled stainless steel discs are washed by continuously lifting them up and	

Assay I	down into the wash liquor, 50 times per minute. After wash, the stainless steel discs are rinsed with running deionized water.

Test conditions for CIP using Assay I with a wash liquor comprising enzyme mix 2: SEQ ID NO: 2, SEQ ID NO: 4 and SEQ ID NO: 6 in 50 mM carbonate buffer, pH 10.

Buffer	50 mM Carbonate buffer, pH 10	
Benchmark	1% NaOH	
Controls	Deionized water and 50 mM carbonate buffer, pH 10	
Enzymes	Enzymes	
Enzyme dosage	SEQ ID NO: 2	11.2 mg EP/200 ml wash liquor
	SEQ ID NO: 4	3.6 mg EP/200 ml wash liquor
	SEQ ID NO: 6	0.4 mg EP/200 ml wash liquor
	Total enzyme dosage	15.2 mg EP/200 ml wash liquor
Wash liquor volume	200 ml	
Temperature of wash liquor	Room temperature	
Cleaning time	8 minutes	
Rinse time	15 seconds	
Test system, Assay I	Soiled stainless steel discs are washed by continuously lifting them up and down into the wash liquor, 50 times per minute. After wash, the stainless steel discs are rinsed with running deionized water.	

The washed and rinsed stainless steel discs were subsequently dried at 65°C overnight and the cleaning performance determined gravimetrically by measurement of the final weight of the stainless steel discs and evaluated in proportion to the initial weight of the soil.

The results for the cleaning performance are shown in the tables below. The results are normalized so that the performance of the benchmark, 1% NaOH, is set to 100.

Wash liquor	**Cleaning performance**
1% NaOH	100
Enzyme mix 1	99
1% H_3PO_4	86
50 mM carbonate buffer, pH	97

10	
Deionized water	92

These results show that the enzyme mix 1 of the example exhibits a wash performance comparable to the benchmark, 1% NaOH.

Wash liquor	**Cleaning performance**
1% NaOH	100
Enzyme mix 2	97
1% H_3PO_4	86
50 mM carbonate buffer, pH 10	97
Deionized water	92

These results show that the enzyme mix 2 of the example exhibits a wash performance comparable to the benchmark, 1% NaOH. Carbonate buffer exhibits a cleaning performance on level with the enzymatic mix 2 investigated in the present example.

Example 2

10 HL fermentations vessels were soiled and cleaned according to assay II. The volume of the wash liquor was 10% of the total volume of the fermentation vessel.

The fermentation vessels were cleaned using the wash liquors as described in the table below:

		1% NaOH	1% H_3PO_4	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 4	SEQ ID NO: 6
Fermentation vessel 1	Wash liquor I	+	-	-	-	-	-
Fermentation vessel 2	Wash liquor II	-	+	-	-	-	-
Fermentation vessel 3	Wash liquor III	-	-	-	-	-	-
Fermentation vessel 4	Wash liquor IV (enzyme	-	-	77 mg EP/L cleaning	-	18 mg EP/L cleaning	2 mg EP/L cleaning solution

	mix 1 in 50 mM carbonate buffer, pH 10)			solution		solution	
Fermentation vessel 5	Wash liquor V (enzyme mix 2 in 50 mM carbonate buffer, pH 10)	-	-	-	56 mg EP/L cleaning solution	18 mg EP/L cleaning solution	2 mg EP/L cleaning solution

After 60 minutes of cleaning the fermentation vessels were rinsed with tap water by five flushes each for five seconds with 5 seconds pause in between each flush.

The cleaning performance of the individual wash liquors were determined by rating as described in Assay II and shown in below table:

Wash liquor I (1% NaOH)	1	Clean
Wash liquor II (1% H_3PO_4)	2	Lightly soiled
Wash liquor III (50 mM Carbonate buffer, pH 10)	4	Very soiled
Wash liquor IV (enzyme mix 1 in 50 mM carbonate buffer, pH 10)	1	Clean
Wash liquor V (enzyme mix 2 in 50 mM carbonate buffer, pH 10)	3	Soiled

As demonstrated from the rating of the cleaning performance of the production equipment, when tested in pilot scale with installed CIP-system and spray head in top of the fermentation vessel, the enzyme mix 1 in 50 mM carbonate buffer, pH 10 exhibits once again a cleaning performance comparable to the benchmark wash liquor, 1% NaOH. Furthermore, carbonate buffer exhibits a very poor cleaning performance and the cleaning performance observed for the enzymatic mix can therefore be assigned to the activity of the enzymes and not the carbonate buffer which the enzymes are applied in. On the contrary, when tested in pilot scale the enzyme mix 2 exhibits poor cleaning performance. The poor cleaning performance was due to significant foam formation, and the use of enzyme mix 2 for CIP cleaning is therefore not recommended due to its low foaming properties. Enzyme mix 1 showed good performance and no problems with foam occurred during the cleaning process.

Example 3

The foaming properties of 50 ml of a wash liquor (with or without enzyme), when in contact with fermentation soil, were evaluated as described in assay III above.

The foaming properties of the washing liquors described in the table below were investigated:

Wash liquor I	1% (wt.) NaOH
Wash liquor II	50 mM Carbonate buffer, pH 10
Wash liquor III (SEQ ID NO 1 in 50 mM Carbonate buffer pH 10)	1100 mg EP/L 50 mM Carbonate buffer, pH 10
Wash liquor IV (SEQ ID NO 2 in 50 mM Carbonate buffer pH 10)	800 mg EP/L 50 mM Carbonate buffer, pH 10

After application of the milk frother for 2 minutes, the height of the generated foam was measured. Between 7 and 10 measurements were performed for each wash liquor. The results are shown in the table below:

	Foam height in cm
Wash liquor I (1 wt.% NaOH)	1.8 ± 0.6
Wash liquor II (50 mM Carbonate buffer, pH 10)	1.1 ± 0.5
Wash liquor III (SEQ ID NO 1 in 50 mM Carbonate buffer pH 10)	0.2 ± 0.08
Wash liquor IV (SEQ ID NO 2 in 50 mM Carbonate buffer pH 10)	1.6 ± 0.8

By comparison of wash liquor III and IV it can be seen that the foam formation is reduced significantly when SEQ ID NO: 1 is used compared to SEQ ID NO: 2. In fact, approximately 90% less foam is formed when SEQ ID NO: 1 is applied compared to SEQ ID NO: 2. When comparing with wash liquor II (carbonate buffer), application of SEQ ID NO: 1 in carbonate buffer results in approximately 85% less foam being produced, whereas application

of SEQ ID NO: 2 in carbonate buffer results in approximately 50% more foam being generated. Also, by comparison of the amount of foam being generated in 1% (wt.) NaOH and carbonate buffer containing SEQ ID NO: 1, a significant foam reduction is obtained by use of SEQ ID NO: 1 in carbonate buffer. There is on the contrary no difference in the amount of foam being generated in 1% (wt.) NaOH and carbonate buffer containing SEQ ID NO: 2.

The results clearly demonstrate the advantages in applying SEQ ID NO: 1 instead of SEQ ID NO: 2, when limited foam formation is the objective.

CLAIMS

1. Use of an enzyme for cleaning-in-place of process or production equipment, wherein the enzyme is selected from the group consisting of:
   a. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus licheniformis*,
   b. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus halodurans*,
   c. an enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1; and
   d. an enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 268 of SEQ ID NO: 3.

2. Use of an enzyme according to claim 1, wherein the process or production equipment is process or production equipment used in food industry, pharma industry and biotech industry.

3. Use of an enzyme according to any of the preceding claims, wherein the enzyme has low foaming properties.

4. Use of an enzyme according to any of the preceding claims, wherein the use further comprises using one or more enzymes selected from: hemicellulases, peroxidases, proteases, cellulases, xylanases, lipases, phospholipases, esterases, cutinases, pectinases, mannanases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, pullulanases, tannases, pentosanases, malanases, ß-glucanases, arabinosidases, hyaluronidase, chondroitinase, laccase, chlorophyllases, amylases, perhydrolases, peroxidases or mixtures thereof.

5. Use of an enzyme according to any of the preceding claims, wherein the use further comprises using an enzyme having amylolytic activity and an enzyme having cellulolytic activity.

6. Use according to claim 5, wherein the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%

identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having amylolytic activity having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 483 of SEQ ID NO: 4; and an enzyme having cellulolytic activity having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 287 of SEQ ID NO: 6.

7. Detergent composition for cleaning-in-place which detergent composition comprises:
    a. From 0.001 wt. % enzyme protein to 0.16 wt. % enzyme protein of an enzyme having proteolytic activity, which enzyme is selected from the group consisting of:
        i. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus licheniformis*,
        ii. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus halodurans*,
        iii. an enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and
        iv. an enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 268 of SEQ ID NO: 3,
    b. optionally from 0.01 wt. % to 2 wt. % of a surfactant,
    c. from 0.5 wt% to 2 wt% of a carbonate source, and
    d. water.

8. Composition according to claim 7, wherein the composition further comprises one or more enzymes selected from: hemicellulases, peroxidases, proteases, cellulases, xylanases, lipases, phospholipases, esterases, cutinases, pectinases, mannanases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, pullulanases, tannases, pentosanases, malanases, ß-glucanases, arabinosidases, hyaluronidase, chondroitinase, laccase, chlorophyllases, amylases, perhydrolases, peroxidases or mixtures thereof.

9. Composition according to any of claims 7-8, wherein the composition further comprises an enzyme having amylolytic activity and an enzyme having cellulolytic activity.

10. Composition according to any of claims 7-9, wherein the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having amylolytic activity having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and an enzyme having cellulolytic activity having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6.

11. Composition according to any of the preceding composition claims, which composition comprises:
    a. From 0.008 wt% enzyme protein to 0.16 wt% enzyme protein of an enzyme having proteolytic activity, which enzyme is selected from the group consisting of:
        i. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus licheniformis*,
        ii. an enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and
    b. from 0.5 wt. % to 1 wt. % of a surfactant,
    c. from 0.5 wt% to 1 wt% of a carbonate source, and
    d. water.

12. A method for cleaning-in-place, wherein a wash liquor is circulated in a production or a process equipment and wherein the wash liquor comprises a carbonate source and an enzyme selected from the group consisting of:
    a. An enzyme having proteolytic activity, which enzyme is derived from *Bacillus licheniformis*,
    b. an enzyme having proteolytic activity, which enzyme is derived from *Bacillus halodurans*,

c. an enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and

d. an enzyme having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-268 of SEQ ID NO: 3.

13. Method according to claim 12, wherein the method comprises cleaning-in-place of production or process equipment, which method comprises the following steps :

a. Optionally pre-rinse by circulating water in the equipment and subsequently drain water;

b. circulate in the equipment a wash liquor (i) or a wash liquor (ii) for a sufficient period of time:

i. wash liquor comprising the detergent composition of any of claims 7-11; or

ii. wash liquor comprising the enzyme having proteolytic activity;

c. optionally stop circulation under step b and allow the process or production equipment to soak in the wash liquor;

d. drain the wash liquor;

e. rinse by circulating water in the equipment and subsequently drain water;

wherein steps a), (b), (c), (d) or (e) are carried out one, two or three times.

14. Method according to any of the claims 12-13, wherein wash liquor further comprises one or more enzymes selected from: hemicellulases, peroxidases, proteases, cellulases, xylanases, lipases, phospholipases, esterases, cutinases, pectinases, mannanases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, pullulanases, tannases, pentosanases, malanases, ß-glucanases, arabinosidases, hyaluronidase, chondroitinase, laccase, chlorophyllases, amylases, perhydrolases, peroxidases or mixtures thereof.

15. Method according to any of the claims 12-14, wherein the method further comprises using an enzyme having amylolytic activity and an enzyme having cellulolytic activity.

16. Method according to any of claims 12-15, wherein the enzyme having proteolytic activity has at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1 to 274 of SEQ ID NO: 1, and is used together with an enzyme having amylolytic activity having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-483 of SEQ ID NO: 4; and an enzyme having cellulolytic activity having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identity, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to amino acids 1-287 of SEQ ID NO: 6.