ABSTRACT OF THE DISCLOSURE

Detergent compositions, usually containing surfactant and/or other conventional cleansing ingredients, comprising a soluble polymer-enzyme product wherein the enzyme is covalently bound, preferably one wherein the enzyme moiety comprises a protease, and especially such compositions embodying a plurality of polymer-enzyme products or a polymer-plural enzyme product, or combinations thereof, thereby imparting an even greater operative range of enzymatic substrate and pH activity to the detergent composition; production and use of such compositions.

BACKGROUND OF INVENTION

(1) Field of invention

Detergent products. Enzyme-containing.

(2) Prior art

Detergent compositions containing surfactants of various types, including cationic, anionic, or nonionic, have long been known. These usually comprise the surfactant plus a detergent builder, which has in the past comprised an inorganic phosphate. Of late, the phosphate builder has been replaced in whole or part by more acceptable materials such as NTA (alkali salts of nitritolactic acid), EMA (ethylene-maleic anhydride copolymer), and like materials. Also, rather recently, enzymatic materials have been added to such detergent compositions to improve the detergency of the composition through their enzymatic activity.

Enzymes can be used in a soaking or prerewash product designed for preparing the dirty item for greater detergency when subjected to conventional washing, or they can constitute a component included in a detergent formula containing the conventional cleansing ingredients. Enzymes suitable for such purposes in laundering are usually in the form of a fine powder.

Enzymes have been used as cleaning co-adjutants for many years. As long ago as 1915 Rohm discovered that fabrics could be cleaned more easily and at lower temperatures when pretreated with fats and protein-digesting enzymes. Rohm, German Patent 285,923 (May 15, 1915).

Later, in 1932, enzymes were used in a soap composition which had improved cleaning powers. See Frelinghuyzen, U.S. Pat. 1,882,279 (Oct. 11, 1932). Enzymes assist in laundering by attacking dirt and stains found on or in dirty materials, e.g., fabrics, dishes, floors, walls, and the like. Dirty matter and stains are broken down or altered by this attack, making them easier to remove during subsequent washing.

Such enzyme-containing compositions have presented numerous problems and still leave much to be desired in practice. For example, the enzyme material is reactive in aqueous solution and consequently must be present in the detergent composition in dry form. Originally, the enzymes were sprayed directly upon or dry-mixed with surfactant, but this was found unsatisfactory in practice due to inactivation of the enzyme because of reaction between the enzyme and the surfactant upon which it was sprayed. This disadvantage has been obviated by provision of soluble particulate solid materials, such as sodium sulfate or the like, upon which the enzyme has been sprayed and thereafter dried. Lactose, calcium sulphate, and similar materials, as well as some of the usual bulking materials, have also been employed as enzyme carriers. This practice, however, has required the additional expense of the carrier material and in spraying and drying the enzyme material thereupon, or blending it therewith, but has resulted in an increased stability of the enzyme and compositions containing the same. Nevertheless, such compositions are still characterized by important disadvantages, such as a limited shelf life due to inactivation or deterioration of the enzyme and a limited activity of the enzyme in aqueous solution when the detergent is employed in practice. Such products are also characterized by an undesirable color which is imparted to the composition due to colored impurities which are normally inherent in the enzyme materials as presently available and employed.

Insoluble combinations of polymers, such as EMA, and an enzyme, in which the enzyme is covalently bound, are known. However, we have found that such insoluble products are unsuitable for incorporation in detergents because of their lowered enzymatic activity in aqueous solution against other solid, semisolid, or like materials, the removal of which is desired, and because of their insolvency, which we have found to permit residues of the polymer-enzyme product, with lingering enzymatic activity, on the item, object, or subject treated therewith. Only one instance of a reported soluble polymer-enzyme product is known to us. The product involved a different type of polymer. It was never suggested for employment in a detergent composition or for any other purpose. Contrary to the findings of Mitzi et al., Nature 189, 576 (1961), who reported that there was no change in the pH-activity characteristics of their particular trypsin and chymotrypsin polymer products from that of the native enzymes, we have found that our particular polymer-trypsin and chymotrypsin polymer products, which can be employed in the compositions of the invention, are characterized in the form of anionic-polymer-enzyme products by a striking pH shift and flattening out of their pH activity curves into the upper alkaline regions, making these products all the more suitable for use in alkaline detergent compositions, today certainly one of the most important areas of the detergent field. Thus, our soluble anionic trypsin-polymer and chymotrypsin-polymer products are unpredictably more useful in the alkaline detergent areas because of their unpredictable pH activity characteristics. This is even more unexpected since the substrate should have more complete accessibility to the attached enzyme which is now not associated with a crosslinked system, as is characteristic of insolubles. The other soluble polymer-enzyme products employed by us as enzymatically-active components of detergent compositions, especially those wherein the enzyme moiety is of microbiological origin, are even more distant from known polymer-enzyme products, and no such soluble polymer-enzyme products as employed by us according to the present invention, so far as we are aware, have been hitherto known.

Again surprisingly, the stability and duration of activity of our novel soluble polymer-enzyme products is still found to be much increased relative to the native enzyme. This increased stability may be the result of several possible factors, but it cannot be readily explained at this time. At any rate, these soluble products are remarkably stable against autoysis and denaturation, even in solution and, perhaps due to this stability or other factors, exhibit a substantially prolonged enzymatic activity in use, even in aqueous solution. Thus, according to the invention,
novel detergent compositions embodying novel water-soluble polymer-enzyme products are provided.

SUMMARY OF THE INVENTION

Detergent compositions embodying soluble polymer-enzyme products, in which the enzyme is covalently bound, are the essence of the present invention. Preferably the enzyme moiety of the polymer-enzyme product comprises a proteinically active water-soluble polymer-enzyme product, a preferred embodiment of the invention involves incorporation of a plurality of polymer-enzyme products into the detergent composition, and a still more preferred embodiment involves employment of a polymer-plural enzyme product having a plurality of enzymes covalently bound therein, thereby increasing the effective pH and substrate range of the composition substantially. The compositions of the invention are characterized by improved color, improved stability, improved shelf life, prolonged activity of the enzymatically active component of the composition in solution, and increased economy due to the necessity of employing reduced amounts of the enzyme material due to its prolonged activity and stability, and reduced amounts of usual detergent bulking agent due to increased bulk of the enzyme-polymer component.

OBJECTS

The provision of a detergent composition having any or all of the foregoing enumerated advantages, and methods of making and using the same, is accordingly an object of the present invention. Another object of this invention is to provide a detergent composition containing an enzymatically active water-soluble polymer-enzyme product. Other, more specific, objects of this invention are to provide a detergent composition containing a soluble polymer-enzyme product wherein the enzyme is covalently bound; such compositions wherein the enzyme moiety of the enzyme-polymer product comprises a protease; and in general detergent compositions containing such polymer-enzyme products whereby the stability and useful life of the enzymatically active component both in storage and in action is greatly improved. Still a further object involves the provision of such compositions embodying a plurality of enzyme products, or, preferably, a polymer-plural enzyme product, or combinations thereof. Another object of this invention is to provide a novel means of incorporating enzymes into a detergent composition which achieves greater enzyme stability, prolonged enzyme activity, and permits economy through the use of smaller amounts of enzyme material and detergent bulking agent. Other objects will become apparent from the detailed specification hereinafter given, and still others will be obvious to one skilled in the art. All the parts, percentages and relative proportions given herein are by weight unless otherwise indicated.

GENERAL DESCRIPTION OF INVENTION

We have now found that compositions incorporating soluble polymeric products comprising polymer chains, e.g., of the EMA type, having an enzyme covalently bonded thereto, not only retain their enzymatic activity over long periods, especially in the dry state, but that the polymer-enzyme products which are utilized therein are soluble in aqueous solutions to a sufficient extent that a high order of enzymatic, e.g., proteolytic, detergency is imparted to compositions containing the same. The polymer-enzyme materials employed have the further advantage that they do not impart the usual coloration which characterizes detergent compositions containing enzymes in unbound form. For this reason, no coloring or masking dyes need be added. In addition, compositions comprising these materials are highly stable, have a substantially improved shelf life and a significant prolongation of enzymatic activity in aqueous solution when employed as detergents. Moreover, these compositions are characterized by a substantial freedom from the objectionable odor which characterizes compositions containing unbound enzymes, due to the fact that they may be incorporated into the detergent composition as a relatively pure form in which the enzymes themselves cannot be provided or employed. Therefore, perfumes or masking agents are not necessary. Although incorporation of polymer-enzyme product in a detergent composition is of the essence of the invention, we have found that it is also possible and advantageous to employ mixtures of a plurality of such polymer-enzyme materials or, preferably, a polymer-enzyme product which has covalently bound therein not only a single enzyme, e.g., an alkaline protease, but also one or more additional enzymes which are not the same but which, rather, operate on different substrates and at different pH ranges, thus to extend the operative range of enzymatic activity of the detergent composition still further. For example, a particularly advantageous polymer for incorporation into detergent compositions according to the invention involves a water-soluble polymer-enzyme product comprising a polymer which has covalently attached to the chains thereof not only an alkaline protease which is operative at a pH of 8 to 11, or even higher, but also an additional enzyme or enzymes, such as a neutral protease and amylase or lipase as well. Combinations of polymer-enzyme and polymer-plural enzyme products are also most advantageously employed.

Definitions

As used herein, the term “detergent” means any cleansing or cleaning agent, having as a synonym the word “abstergent.” It can be any of various types, as already well established in the art. For example, it can be of a type having a formula R—COOM, representing soaps, which are generally the alkali or metal salts of fatty acids, usually higher fatty acids, or the formula

\[ \text{SO}_3\text{O}—\text{R}—\text{COOM} \]

representative of turkey red oils, or sulfonated fatty acids, usually higher fatty acids, or \((\text{SO}_4\text{O})_2\text{R}—\text{COOM}\), representative of monopole soap or highly sulfonated fatty acids, usually higher fatty acids, or the formula R—SO_4M, representative of guardinos, or sulfonated fatty alcohol, usually higher fatty alcohols, sulfonated alkylbenzenes, e.g., sodium dodecylbenzenesulfonate, and any other cleansing or cleaning agent even though of a formula different from those given in the foregoing, including nonionic detergents such as ethoxylates of long-chain alcohols and alkylphenols, as well as nonionic, anionic, and amphoteric detergents, as further considered hereinafter. Of course, variations within the established formulas and groupings given are also well-established in the art and are intended to be included within the definition. These organic detergent materials are often known as surface active agents. They are more fully described in the textbook by Schwartz and Perry, entitled Surface Active Agents, Interscience Publishers, Inc., New York, N.Y. (1949). A detergent composition, therefore, is any composition, preferably in the dry state, having any type of detergent included therein.

Thus, representative detergents include cleaning compounds, whether for home use or use in industrial systems, including by way of illustration but not limitation retail products such as liquid and bar soaps, laundry detergents, dishwashing compounds, hard surface cleaners, all purpose cleaners, water softeners, detergent extenders, preswash or soaking compositions, and the like.

“EMA” is a polymer of ethylene and maleic anhydride. Polymers of this type and of great value according to the present invention.

"EMA type" polymer is defined hereinafter.

"EMA-enzyme" or "EMA/enzyme" is a copolymer of ethylene and maleic anhydride having enzyme covalently bonded thereto. The product is the same whether the enzyme is reacted directly with an anhydride group of
the ethylene-maleic anhydried copolymer or with a carboxyl group of the ethylene-maleic anhydride copolymer, whether or not using an intermediate activating mechanism for carboxyl groups of the polymer. Anhydride groups not participating in the reaction by which the product is produced in aqueous medium are present in the product as carboxyl or anhydride groups. Such non-participating groups may, however, be converted to amide, imide, ester, and cetera, groups, as can be present in EMA-type polymers, as hereinafter defined.

"Water-insoluble" means that the product concerned does not dissolve in water or aqueous solutions, although it may have such characteristics as degree of swelling due to water solution, even to the extent of existence in gel form. "Water-soluble" means not water-insoluble, and is further defined hereinafter.

The enzymatically-active products used in the detergent compositions of the invention are water-soluble polymer-enzyme products wherein the enzyme is bound covalently through a group which is non-essential for enzymatic activity to a polymer (a) comprising chains of carboxylic acid or carboxylic acid anhydride units or (b) comprising units of carboxylic acid or carboxylic acid anhydride groups separated by carboxylic chains of at least one and not more than twenty carbon atoms. Said carbon chains being part of a unit which contains a maximum of eighteen carbon atoms, said polymer chains ordinarily being formed by polymerizing polymerizable acids or anhydrides or by copolymerizing a polymerizable acid or anhydride with another polymerizable monomer, and preferably wherein the starting acid or anhydride and any additional polymerizable monomer are unsaturated and such polymerization or copolymerization comprises addition type polymerization or copolymerization involving such unsaturation.

Process for preparing polymer-enzyme products

Polymer-enzyme derivatives can be prepared by reacting the crystalline or crude enzyme or mixture of enzymes with the polymer in solution, resulting in formation of a polymeric product in which the enzyme is covalently bound. Since an anhydride or carboxyl is present in the polymer, e.g., an EMA-type polymer, covalent bonding of the enzyme to the polymer may be effected directly through reaction or coupling with an anhydride group or with a carboxyl group of a polymerizable monomer, and preferably wherein the starting acid or anhydride and any additional polymerizable monomer are unsaturated and such polymerization or copolymerization comprises addition type polymerization or copolymerization involving such unsaturation.

When carried out in this manner, the results are production of the desired active polymer-enzyme derivative, but degree of activity imparted to the polymeric product is sometimes lower than desired, possibly due to partial inactivation of the enzyme during the process. Resort may often advantageously be had to employment of a mixed solvent system, the solvent in which the enzyme is at least partially soluble, usually in an amount up to about 50% by volume. Dimethylsulfoxide (DMSO) is especially suitable as solvent together with water or aqueous buffer solution in a mixed solvent system. Using such a mixed solvent system, the desired active enzyme polymer product is ordinarily obtained in higher yields and conversions to desirably active product, and introduction of desirably high amounts of enzyme activity into the polymer molecule is generally less difficult.

As stated, the polymer in such reaction contains carboxyl or anhydride linkages, especially where the enzyme contains an amino hydroxyl (including phenolic hydroxyl), or sulfhydryl group not essential for its enzymatic activity. The polymer is preferably EMA or an EMA-type polymer, but it can be any of those types herein disclosed for coupling or reaction with an enzyme, and in any event it is adapted to effect covalent bonding with the enzyme to produce an enzyme-polymer product either directly or indirectly using an activating agent. Inasmuch as the enzymatic activity of the starting enzyme is desired to be retained in the final product, it is of course firstly necessary that bonding of the enzyme to the polymer be through a group which will not result in inactivation of an active site in the enzyme molecule. Among the various activating agents which may be mentioned, besides amino and sulfhydryl, also hydroxyl (including phenolic hydroxyl), carboxyl and imidazolyl. Such groups are present in free or unbound form in active portions of enzyme molecules, as in a lysine, cysteine, serine, threonine, histidine, or tyrosine moiety of an enzyme molecule, where the particular moiety in question is not considered essential for enzymatic activity, either catalytic in nature or for substrate binding. Therefore, attachment to the polymer molecule is through reaction of the polymer with such group so as to avoid inactivation of the enzyme during attachment to the polymer molecule. Generally this is accomplished by ester, thioester, or disulfide group, such as formed by the carboxyl or anhydride with an amine or hydroxyl group in a non-essential moiety of the enzyme protein chain. Amides are conventionally formed by reacting pendant amino groups of the enzyme with carboxylic anhydride groups on the carrier polymer in water, in aqueous enzyme media, or in mixed solvents. Amides, imides and esters are readily formed by activating carboxyl groups of the polymer, and reacting them with respective hydroxyl, amine or mercaptan groups on the other reactant. Such activation may be effected using various carboximidates, carbodiimidoazoles, Woodward’s or Sheehan’s reagent, or the like, to form highly active intermediates capable of reacting with groups in the enzyme under mild conditions, the latter favoring retention of enzymatic activity.

The polymer selected for such reaction can therefore be said to be adapted to couple or react with the enzyme, either directly or indirectly through use of an activating agent, as already indicated, and in any event to effect covalent bonding with the enzyme. The attachment procedures given are conducted by techniques adapted to include any requisite protection for the enzyme, which may include a reversible blocking of the enzymatically active site or sites, as for example in the case of papain, or use of mercuripain or zinc papain may be employed as an intermediate for reaction with the polymer in order to effect greater yields upon attachment, the protecting atoms being removed subsequent to the attachment reaction.

General procedure for solubilization

In order to achieve high yields of water-soluble enzyme-polymer products, it is desirable to avoid crosslinking which results in insolubilization.

To prepare water-soluble enzyme-polymer derivatives, therefore, the reaction is preferably performed under substantially non-crosslinking conditions. The undesired crosslinking can be reduced by performing the attachment reaction in high dilution such that fewer reactions occur between several polymer molecules and a single enzyme molecule. Alternatively, high ratios of enzyme to polymer favor reaction of several enzyme molecules with a single polymer molecule. This, therefore, results in an agglomerated enzyme/polymer system which maintains the desired solubility of the individual enzyme molecules. An additional way of favoring "solubilization" formation is to run the reaction at high ionic strength to decrease aggregation of the native protein. While such procedures as described above are often desirable, it is not always necessary to use dilute solutions or high enzyme/polymer ratios to cause formation of soluble enzyme/polymer derivatives.
The term “water-soluble” means that the product concerned dissolves in water or aqueous solutions. As usual, however, this does not mean that the product dissolves completely at all concentrations or at all pH's. On the other hand, these water-soluble products are characterized by being soluble at a variety of concentrations and pH's, and they are generally soluble at pH's of 7 or greater.

In their soluble form, the polymer-enzyme products of the invention are characterized by fundamentally the same enzymatic action as the parent native enzyme, but have all of the advantages which are attendant upon applicability in solution or suspension form together with increased stability and prolonged activity. In addition, because of their polymeric form, even though soluble, the polymer-enzyme products of the invention are separable from native enzyme or substrates, as well as impurities and coloring matter of an undesired nature, by normal separation procedures such as centrifugation, electrophoresis, or chromatography.

Polymere reactant

In its broadest context, the polymer to which the enzyme is coupled or reacted contains carboxyl or anhydride linkages, especially where the enzyme contains an amino group, as hydroxyl or sulfhydryl group not essential for its enzymatic activity. The polymer may be EMA or an EMA-type polymer, or be any of those types disclosed herein for coupling or reaction with an enzyme, and in any event it is adapted to couple or react with the enzyme to effect covalent bonding and production of the desired soluble enzyme-polymer product.

Since covalent bonding is desired, it is understood that the carrier polymer is tailored to contain at least one reactive site for each polymer molecule with which the enzyme can react, either directly or indirectly, to produce a covalent bond. According to the instant invention, this reactive site (or sites) is preferably a carboxyl or carboxylic anhydride group.

The polymeric reactant, according to the invention, may be defined broadly as follows: a polymer (a) comprising chains of carboxylic acid or carboxylic acid anhydride units, or (b) comprising units of carboxylic acid or carboxylic acid anhydride groups separated by carbon chains of at least one and not more than four carbon atoms, said carbon chains being part of a unit which contains a maximum of eighteen carbon atoms, said polymer chains being formed by polymerization of polymeric acid or anhydrides, or by copolymerizing a polymeric acid or anhydride with another copolymerizable monomer, and preferably wherein the starting acid or anhydride and any additional copolymerizable monomer are unsaturated and such polymerization or copolymerization comprises addition type polymerization or copolymerization involving such unsaturation.

Among the polymers suitable for the practice of the instant invention, polymeric polyelectrolytes having units of the formula

\[ (Q)_p - W - (NR'R')_p \]

wherein \( p \) is zero or one, wherein \( R \) is selected from the group consisting of alkyl, phenylalkyl, or phenyl, in each case of 1 to 18 carbon atoms, wherein \( R' \) is H or R, wherein Q is oxygen or -NR-, and wherein W is a bivalent radical preferably selected from lower-alkylene, phenyl, phenylalkyl, phenylalkyloxyphenyl, and alkylphenylalcohol having up to 20 carbon atoms, X and Y taken together can be an oxygen atom, and at least one of X and Y being hydroxyl or X and Y together constituting an oxygen atom, are preferred. Many of these polymers are commercially available and others are simple derivatives of commercially available products which can be readily prepared either prior to or simultaneously with the enzyme attachment reaction, or produced as a minor modification of the basic polymer after attachment.

Such polymers containing the above-described EMA-type units are hereinafter referred to as an "EMA-type polymer.

Since enzyme molecules have an extremely high molecular weight, even if the polymeric unit exemplified as usable for attachment of the enzyme occurs only once in a polymer chain, for example, once in every several hundred units, reaction of the enzyme with this unit will result in an enzyme-polymer product having substantial enzymatic activity and one wherein the enzyme moiety constitutes a substantial portion of the molecular weight of the polymer-enzyme product. If more than one of the exemplified units is present, multiple attachments can be achieved with increased enzymatic activity of the product. As pointed out hereinafter, preferably the units of the formula given are recurring, \( n \) being at least 8. When the units are recurring, the symbols in the various recurring units do not necessarily stand for the same thing in all of the recurring units. Moreover, where the units are recurring, some of the \( X \) and \( Y \) groups may have meanings besides hydroxyl or oxygen. For example, some, but not all, of them may be present in the form of inside groups, that is, groups in which \( X \) and \( Y \) together are -NR- or -N-W-(NR'R')_p, wherein R, W and R' have the values previously assigned.

A preferred type of polymeric material is the polymer of an olefinically unsaturated polycarboxylic acid derivative with itself or in approximately equimolar proportions with at least one other monomer copolymerizable therewith. The polycarboxylic acid derivative can be of the non-vicinal type, including acrylic acid, acryl anhydride, methacrylic acid, crotonic acid, or their respective polybasic acid derivatives can be copolymers with a plurality of co monomers, in which case the total amount of the co

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A monomer suitable for use with the above functional monomers include a-olefins such as ethylene, propylene, isobutylene, 1- or 2-butene, 1-hexene, 1-octene, 1-decene, 1-dodecene, 1-octadecene, and other vinyl monomers such as styrene, a-methylstyrene, vinyl acetate, vinyl amine, vinyl chloride, vinyl formate, vinyl propionate, vinyl alkyl ethers, e.g., methylvinylether, alkyl acrylates, alkyl methacrylates, acrylamides and alkylacrylamides, or mixtures of these monomers. Reactivity of some functional groups in the copolymers resulting from polymerization of the above-mentioned other useful functional groups in the formed copolymer, including hydroxy, lactone, amine, and lactam groups.

Any of the said polybasic acid derivatives may be copolymerized with any of the other monomers described above, and any other monomer which forms a copolymer with dibasic acid derivatives can be copolymers with a plurality of comonones, in which case the total amount of the co
monomers will preferably be about equimolar with respect to the polybasic acid derivatives. Although these copolymers can be prepared by direct polymerization of the various monomers, frequently they are more easily prepared by an after-reaction modification of an existing copolymer.

Copolymers of anhydrides and another monomer can be converted to carboxyl-containing copolymers by reaction with water, and to ammonium, alkaline and alkaline earth metal and alkyamine salts thereof by reaction with alkaline metal compounds, alkaline earth metal compounds, amines, or ammonia, either prior to, during, or subsequent to enzyme attachment. Other suitable derivatives of the above polymers include the partial alkyl or other esters and partial amides, alkyl amides, dialkyl amides, phenylalkyl amides or phenyl amides prepared by reacting carboxyl groups on the polymer chain with the selected amines or alkyl or phenylalkyl alcohol as well as amino esters, amino amides, hydroxy amides and hydroxy esters, wherein the functional groups are separated by lower-alkylene, phenyl, phenylalkyl, phenylalkylphenyl, or alkylphenylalkyl, which are prepared in the same manner in each case with due consideration of preservation of enzyme attachment sites as previously stated. Other aryl groups may be present in place of phenyl groups. Particularly useful derivatives are those in which negatively-charged carboxyl groups are partially replaced by amine or amine salt groups. These are formed by reaction of said carboxyls with polyamines such as dimethylamine, dimethylaminoethanol, the former forming an amidine linkage with the polymer and the latter an ester linkage. Suitable selection of the above derivatives permits control of several parameters of performance for the enzyme-polymer products used in the invention.

Representative dibasic acid or anhydride-olefin polymers, especially maleic acid or anhydride-olefin polymers, of the foregoing type (EMA-type) are known, for example, from U.S. Pat. 2,378,629, 2,396,785, 3,157,595 and 3,340,680. Generally, the copolymers are prepared by reacting ethylene or other unsaturated monomer or mixtures thereof, as previously described, with the acid anhydride in the presence of a peroxide catalyst in an aliphatic or aromatic hydrocarbon solvent for the monomers but nonsolvent for the interpolymer formed. Suitable solvents include benzene, toluene, xylene, chlorinated benzenes and the like. While benzoyl peroxide is usually the preferred catalyst, other peroxides such as acetyl peroxide, butyryl peroxide, di-tertiary butyl peroxide, lauroyl peroxide and the like, or any of the numerous azo catalysts, are satisfactory since they are solubles in organic solvents. The catalyst preferably contains substantially equimolar quantities of the olefin residue and the anhydride residue. Generally, it will have a degree of polymerization of 8 to 10,000, preferably about 100 to 5,000, and a molecular weight of about 1,000 to 1,000,000, preferably about 10,000 to 500,000. The properties of the polymer, such as molecular weight, for example, are regulated by proportion of the catalyst and control of one or more of the variables such as ratio of reactants, temperature, and catalyst concentration or the addition of regulating chain transfer agents, such as diisopropyl benzene, propionic acid, alkyl aldehydes, or the like. The product is obtained in solid form and is recovered by filtration, centrifugation or the like. Removal of any residual or adherent solvent can be effected by evaporation using moderate heating. Numerous of these polymers are commercially available. Particularly valuable copolymers are those derived from ethylene and maleic anhydride in approximately equimolar proportions. The product is commercially available.

The maleic anhydride copolymers thus obtained have repeating anhydride linkages in the molecule, which are readily hydrolyzed by water to yield the acid form of the copolymer, rate of hydrolysis being proportional to temperature. In view of the fact that the attachment re-
Lipases such as those produced by: *Candida parallipolytica*  
*Aspergillus*  
*Rhizopus delemar*  
*Candida cylindracea* (lipase–MY)  
*Molipase* (*Torula lipase*)

A great many enzymes are known and are suitable for incorporation into the water-soluble polymer-enzyme products used according to the invention. Numerous starting enzymes are available commercially, being obtained from various animal, vegetable and microbial sources. Many enzymes are obtained by microbial fermentation, e.g., production of enzymes by fungi or bacteria, using well-known fermentation processes such as those generally described in Kirk-Othmer, *Encyclopedia of Chemical Technology* 8, 173–204.

The exact activity of the enzyme or enzymes employed as starting material is not critical, providing only that the starting enzyme has the desired activity suitable for the ultimately intended use of the polymer-enzyme product. Various analytical methods are available to determine the activity of enzymes and enzymatically active materials, for example, the protease activity of proteolytic enzymes can be determined by well-known casein digestion methods. According to such tests, a protease catalyzes the hydrolysis of casein in a certain period of time and temperature at a certain pH; the reaction is stopped by the addition of trichloroacetic acid, and the solution is filtered. The color of the filtrate is developed by Folin phenol reagent, and the level of enzyme activity is measured spectrophotometrically in units of casein tyrosine. This method is more fully described in the Journal of General Physiology 30, 291 (1947) and in Methods of Enzymology 2, 33, by Academic Press, N.Y. (1955). Amylase activity is generally determined by the well-known dinitrosalicylic acid method of Bernfeld. Other tests are known and well-documented in the art.

A particularly effective source of mixed enzymes which can be used as starting material is amutated *Bacillus subtilis* organism. The process for producing this organism and enzymes therefrom is described in U.S. Patent 3,031,380. A culture of this *Bacillus subtilis* (strain AM) organism has been deposited with the United States Department of Agriculture, Agricultural Research Service, Northern Utilization Research and Development Division, 1815 N. University St., Peoria, Ill. 61604, and has been assigned No. NRRL B-5411. The enzymatically active material produced by this organism has been found to consist of two proteases, approximately 65–75% neutral protease (activity at pH 7.0–7.5) and about 25–35% alkaline protease (activity at pH of 9 to 10). A significant amount of amylase is also present. There are generally about 700 thousand to about 1.2 million units of neutral protease activity per gram of isolated solids and about 250 thousand to about 400 thousand units of alkaline protease activity per gram as determined by Anson's variation of the Kunitz Casein digestion method.

There are generally about 300 thousand to 350 thousand units of amylase activity per gram as determined by the Belfred method. As pointed out in the cited patent, the relative proportions of protease to amylase will vary depending on the exact conditions of growth of the microorganism, but we have found that the neutral and alkaline protease and the amylase will be produced, at least in some amounts, almost regardless of changes in the culture medium and other conditions of growth of the microorganism. The ratio of the activity of the alkaline protease to the activity of the neutral protease in the starting materials and in the polymer-enzyme product is preferably not greater than about 0.25 to 1.2 to one. Another source of enzymes which can be used as starting material in accord with the present invention is *B. subtilis* strain NRRL 941, and *B. subtilis* strain IAM 1523 (Japanese Culture Col-

Preferred polymers are selected from the group consisting of:

- ethylene/maleic anhydride copolymer,  
- styrene/maleic anhydride copolymer,  
- vinyl methyl ether/maleic anhydride copolymer,  
- vinylacetate/maleic anhydride copolymer,  
- divinyl-ether/maleic anhydride cyclocopolymer,  
- polycle anhydride and polyacrylic anhydride, and  
- cationic derivatives thereof.

and preferred enzymes comprise at least one enzyme selected from the group consisting of:

- alkaline protease, neutral protease, acid protease, amylase of diastase and lipase,  
- acid protease particularly when attached to a cationic or basic polyelectrolyte, and all enzymes preferably being of microbiological origin, and  
- combinations thereof. Such combinations of two or more enzyme-polymer products produce results superior to those obtained when only a single enzyme-polymer product is employed and accordingly represents a preferred embodiment of the process. Use of combinations of a plurality of enzymes in the form of a single polymer-plural enzyme molecule is also contemplated by the present invention and represents another preferred embodiment thereof, inasmuch as a multiplicity of enzymatic activities can in this manner be imparted to the detergent composition at once, in the form of a stable product which is not subject to autoysis as are combinations or mere mixtures of enzymes. For example, a polymer-enzyme product containing a protease and an amylase or diastase and/or lipase, or both neutral and alkaline protease and amylase and/or lipase, has been found especially suitable for use according to the invention and such represents an especially preferred embodiment of the invention. Enzymes other than those named in the foregoing must certainly be present in the molecule of the polymer-enzyme product employed, and will serve its own particular function in breaking down the stain, soil, dirt or the same consisting of its special substrate.

**DESCRIPTION OF THE PREFERRED EMBODIMENTS**

The following preparations and examples are given by way of illustration only, and are not to be construed as limited.

**A) PREPARATION OF WATER-SOLUBLE POLYMER-ENZYME PRODUCTS**

Experimental.—The general procedure employed consisted of allowing cold solutions of enzymes in appropriate buffers to react overnight at 4°C with cold, homogenized polymer, e.g., EMA suspensions. EMA–21 was preferably employed, which had a molecular weight of ca. 20,000–30,000. Other molecular weight polymers may also be used. For example, EMA–11; having a molecular weight of about 2–3,000 of EMA–31, having a molecular weight of about 60,000, may also be employed. Separation of soluble and insoluble adds, after reaction, was achieved by centrifugation in the cold (Sorval SS–3 (TM) centrifuge, ca. 10,000 r.p.m. and 10 min. centrifugation time). The soluble adds were generally exhaustively dialyzed against water in the cold and then lyophilized. Insoluble adds were washed (and cen-
trifuged), usually ten times with cold buffer and five times with cold distilled water and then lyophilized.

The reaction of the polymer with the plurality of enzymes, as in some of the preparations can obviously be carried out stepwise, one enzyme at a time, with or without isolation, or with all enzymes at once. The latter procedure is preferred for reasons of time, convenience, and economy.

Preparations 1-3—Soluble EMA-trypsin (SEMAT)

Trypsin (Worthington Biochemical Co.) was stored in the cold and was used as received. EMA was converted completely to anhydride by heating at 105° C. in vacuo to constant weight (ca. 15 hrs.) and then stored in sealed containers until used. BAEE (benzoylarginine ethyl ester) was obtained from Mann Laboratories and used as received.

SEMAT—In a typical experiment 500 mg. trypsin was dissolved in 15 ml. of cold 0.2 M phosphate buffer, pH 7.5 and 1.0 g. of EMA was homogenized in a Waring blender for ca. 1 minute with 100 ml. cold phosphate buffer, pH 7.5. The solutions were combined and the mixture was stirred in a cold room (4° C.) for 12-15 hours. The mixture was then centrifuged in the cold refrigerator (ca. 4° C.). Trypsin solutions were prepared in similar solutions and subjected to identical conditions as for the SEMAT solutions.

Activity of the samples (SEMAT and trypsin) was made by determining their activities toward hydrolysis of BAEE as measured by the (zero order) rate of reaction. The solutions were initially adjusted to have approximately equal activities or activities based upon equivalent protein contents. Activity was measured as a function of time (days). In the case of purified SEMAT preparation, 65% of the original (BAEE) activity was present after 17 days in solution at room temperature whereas a trypsin solution had less than 10% of the original (BAEE) activity after 8 days in solution at room temperature. In another example an unpurified SEMAT preparation retained ca. 60% of the original (BAEE) activity after 28 days in solution at room temperature.

Kinetic parameters—The kinetic parameters, K_m and V_max for SEMAT and trypsin with BAEE as substrate are given in Table 2.

pH activity profiles.—Activity of SEMAT samples and trypsin were made by determining the rate of hydrolysis of BAEE as measured by the change in absorbance at 235 μu. (Table 3).

<table>
<thead>
<tr>
<th>Table 1—SEMAT PREPARATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEMAT</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>2a</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>3a</td>
</tr>
</tbody>
</table>

* Based on dry weight.

† Crude product chromatographed on Sephadex G-100 (crosslinked dextran; phosphate buffer, pH 7.0); product taken from fractions at void volume.

SEMAT purification.—For general purification ca. 100 mg. of crude SEMAT dissolved in a minimum amount of 10% sucrose/0.2 M phosphate of buffer pH 7.5 and placed on a column (ca. 5 x 40 cm.) of Sephadex G-100 (TM-crosslinked dextran) equilibrated with phosphate buffer, pH 7.5. Elution was followed in the usual manner and fractions were collected. The void volume eluant was collected, diazylized and lyophilized to yield product crude SEMAT.

The integrity of the SEMAT was shown by chromatography of physical mixtures of trypsin and HEMA (hydrolyzed EMA) which gave a void volume fraction which had very low protein content as shown by UV absorption at 280 μm vs. 215 μm and low nitrogen content (less than 0.2%). Disc gel electrophoresis toward both positive and negative poles did not give any migrating and stainable bands with purified SEMAT whereas a mixture of trypsin and HEMA gave a band with Rf approximately identical with native trypsin. SEMAT gave a stain (Amido Schwarz dye) when added to the lower gel which was then polymerized and stained.

SEMAT stability.—SEMAT samples were dissolved in 0.2 M phosphate buffer or in 0.1 M KCl and allowed to stand in solution either at room temperature or in a

<table>
<thead>
<tr>
<th>Table 2—TRYPSIN AND SEMAT CATALYZED HYDROLYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme</td>
</tr>
<tr>
<td>Trypsin</td>
</tr>
<tr>
<td>SEMAT</td>
</tr>
<tr>
<td>SEMAT unpurified</td>
</tr>
<tr>
<td>Trypsin, pH 8.0</td>
</tr>
</tbody>
</table>

* Using 2.6 X 10^-4 M EMA's. SEMAT unpurified.

† Using 2.6 X 10^-4 M EMA's. SEMAT unpurified.

§ Using 2.6 X 10^-4 M EMA's. SEMAT unpurified.

Table 3

<table>
<thead>
<tr>
<th>Soluble EMA/Trypsin percent Maximum BAEE Activity vs. pH (Trypsin Buffers to pH 6 Carbonate/Bicarbonate Buffers &gt; pH 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
</tr>
<tr>
<td>6.5</td>
</tr>
<tr>
<td>7.2</td>
</tr>
<tr>
<td>7.6</td>
</tr>
<tr>
<td>8.0</td>
</tr>
<tr>
<td>8.5</td>
</tr>
<tr>
<td>9.0</td>
</tr>
<tr>
<td>9.5</td>
</tr>
</tbody>
</table>

Unpredictably, the pH optimum of SEMAT extends into the higher pH ranges in contrast with native trypsin.

Preparation 4—Chymotrypsin-EMA

To 240 ml. of cold 0.2 M phosphate buffer pH 7.3, was added 1.050 g. of EMA-21 and the mixture was homogenized for one minute. During this time, 210 mg.
of chymotrypsin (crystalline) was dissolved in 60 ml cold, distilled water. The two solutions were combined and magnetically stirred overnight in a cold room. The mixture was then centrifuged to separate soluble and insoluble systems and the two were then lyophilized overnight. The lyophilized soluble and insoluble systems were exhaustively dialyzed against cold water and dialyzed. Weight soluble chymotrypsin/EMA (SEMAC), 1,441.82 mg. Weight insoluble chymotrypsin/EMA (IEMAC), 792.11 mg.

Chromatography of SEMAC—Using the procedure described in Example 1, 100 mg of crude SEMAC was chromatographed on a Sephadex (TM) G-100 column, equilibrated with 0.2 M phosphate buffer, pH 7.5. Elution gave essentially two peaks, one centered near the void volume (206 ml) and the other extending over a wide volume (ca. 250–350 ml). The two peak eluates were collected separately, dialyzed against cold water and lyophilized. Weight Fraction I, 17.60 mg; Weight Fraction II, 22.75 mg.

Assay of chymotrypsin and SEMAC with ATEE (see infra).—ATEE activities of chymotrypsin and SEMAC (Fraction I) were determined using 0.1 M phosphate buffer, pH 7.4. For assay 100 gamma ATTEE (25 mg in 1.25 ml distilled acetone/trit) was added to 3 ml of phosphate buffer. At zero time, 100 gamma of chymotrypsin solution (0.398 mg. chymotrypsin in 1 ml phosphate buffer) or 100 gamma of SEMAC solution (12.750 mg, SEMAC in 1 ml phosphate buffer) was added and the change in optical density at 237 μm as a function of time was recorded on a Cary Model 14 spectrophotometer. Chymotrypsin activity: 0.186 unit/sec/100 gamma; SEMAC activity: 0.422 units/sec/100 gamma. (Units are arbitrary and are based upon change in optical density per unit time.) This and other assays indicated that this SEMAC sample had approximately one-third-second the activity of native chymotrypsin at this particular pH and ionic strength.

TABLE 4

<table>
<thead>
<tr>
<th>pH</th>
<th>Chymotrypsin SEMAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.6</td>
<td>11 12</td>
</tr>
<tr>
<td>8.1</td>
<td>100 44</td>
</tr>
<tr>
<td>9.0</td>
<td>10 9</td>
</tr>
<tr>
<td>9.8</td>
<td>98 109</td>
</tr>
<tr>
<td>10.2</td>
<td>85 88</td>
</tr>
</tbody>
</table>

Unpredictably, the pH optimum of SEMAC extends into the higher pH ranges in contrast with native chymotrypsin.

Stability studies on chymotrypsin, soluble EMA-chymotrypsin and insoluble EMA-chymotrypsin

Chymotrypsin (2.00 mg.), soluble EMA-chymotrypsin (60.02 mg.) and insoluble EMA-chymotrypsin (30.08 mg.) were each dissolved in 2 ml 0.2 M phosphate buffer, pH 7.5, and allowed to stand at room temperature. Exserase activities were measured over a period of days. Exserase activities were measured by determining the rate of hydrolysis of N-acetyl-L-tyrosine ethyl ester (ATEE) as determined by following the change in absorbance at 237 μm of a solution as a function of time. For assay, 100 gamma of a solution of ATEE (40.06 mg in 2 ml acetone/trit) was added to 3 ml of phosphate buffer and hydrolysis was initiated by addition of 10 gamma of the chymotrypsin or soluble EMA-chymotrypsin solutions and by addition of 25 gamma of the insoluble EMA-chymotrypsin. By this method, after two days at room temperature, chymotrypsin had 60% of its initial activity, the insoluble EMA-chymotrypsin had 97% of its initial activity, and the soluble EMA-chymotrypsin had 99% of its initial activity. After seven days at room temperature, the soluble EMA-chymotrypsin solution had 93% of its initial esterase activity.

Preparations 5 and 6—Acid protease-polyacrylic anhydride and EMA polymers

(5) Coupling of acid protease produced by Aspergillus oryzae to a polyacrylic anhydride polymer, in aqueous buffer medium using the conventional procedure of Preparations 1 and 2 at carrier to enzyme ratios of 1:15 to 3:1, yields soluble polymer-protease derivatives having up to about 50% of each of the original enzymatic activities. In the same manner, the identical enzyme-polymer product is produced from polyacrylic acid, using Woodward’s reagent, N-ethyl-3-phenyl isoazoxazolin-5-sulfonyl, as activator for the carboxyl groups of the polyacrylic acid.

(6) Moreover, direct reaction of the acid protease from A. oryzae with EMA—21 in the manner of the foregoing preparations produces soluble EMA-acid protease having an exceptional activity at acid pHs, the percentage of said proteolytic activity based upon starting native acid protease varying between about 23 and 68%.

Preparation 7—B. subtilis neutral and alkaline proteases and amylase/EMA insoluble and soluble adducts

B. subtilis neutral and alkaline proteases and amylase mixture (250 mg.) is dissolved in 100 ml cold 0.1 M in phosphate and 0.01 M calcium acetate, pH 7.5, and to this solution is added a homogenized mixture of EMA (200 mg.) suspended in 50 ml cold 0.1 M phosphate, pH 7.5. The mixture is stirred overnight in the cold (4°C) and the insoluble material is separated from the supernatant by centrifugation. After washing the solids five times with cold 0.1 M NaCl and twice with water, the material is lyophilized to yield a solid which possesses 32% of the original neutral protease activity, 48% of the original alkaline protease activity, and 62% of the original amylase activity.

The ratio of the activity of the alkaline protease to the activity of the neutral protease in the starting materials and in the polymer-enzyme products is preferably not greater than about 0.25 to 1.2 to one.

Preparations 8 to 25—Other soluble polymer-enzyme products

The following additional soluble polymer-enzyme products are prepared in accord with the procedure of Preparation 1. The percentages when given are the percentages of enzymatic activity in the soluble polymer-enzyme product compared with the activity of the starting native enzyme.

(8) Lipase-EMA: 45%.
(9) Amylase-EMA: 72%.
(10) B. subtilis neutral and alkaline protease-EMA soluble adducts: 47%, 59%.
(11) B. subtilis neutral and alkaline protease lipase/EMA soluble adducts: 43%, 56%, 17%.
(12) Neutral protease-EMA.
(13) Alkaline protease-EMA: 35%.
(14) Pepsin-EMA: 10%.
(15) EMA-papain: 15–40%.
(16) EMA-zinc papain: 10%.
(17) Lipase-tyrroly maleic anhydride copolymer: 20%.
(18) Alkaline protease-vinyl methyl ether/maleic anhydride copolymer: 50%.
(19) Diastase-vinyl acetate/maleic anhydride copolymer: 60%.
(20) Amylase/lipase/alkaline protease-divinyl ether/maleic anhydride cyclocopolymer; 50%.
(21) Chymotripsin-polyaminoacrylic anhydride polymer; 70%.
(22) Trypsin-polyaminoacrylic anhydride polymer; 70%.
(23) Alkaline protease/neutral protease-polyaminoacrylic anhydride polymer; 70%.
(24) Acid protease - dimethylaminopropylimide or amide of EMA.
(25) Alkaline protease/neutral protease-diethylamino propyl amide or imide of EMA.
(26) Alkaline protease-diethylaminopropanol ester of EMA.

(B) PREPARATION OF DETERGENT COMPOSITIONS

Examples 1 to 4—Typical detergent compositions containing polymer-enzyme products

Typical U.S. detergents are formulated as all-purpose, heavy-duty detergents, either as high, medium or low sudsers. The following typical chemical compositions are prepared according to the following specifications:

To each of the foregoing compositions is added 2% by weight of the soluble polymer-enzyme product of each of the foregoing preparations (28 separate compositions). Combinations of these 28 polymer-enzyme products are also employed.

The compositions are dry mixed and packaged and are stable, colorless, odorless, and highly enzymatically active even after long periods of storage. They are also totally active in use and extremely effective in removing ordinarily difficultly removable stains of various types from the materials cleansed therein by enzymatic action.

Particularly effective compositions are those which embody the polymer-enzyme products of the foregoing preparations, reference being made to the preparation numbers:

An all-purpose high suds, particularly suitable for use as a household laundry detergent, contains about 1-5%, e.g., 2%, of a polymer-enzyme composition set forth in A-T preceding, and otherwise has the following formulation:

<table>
<thead>
<tr>
<th>Percent by weight</th>
<th>All-purpose</th>
<th>All</th>
<th>Heavy duty</th>
<th>All</th>
<th>Solder</th>
<th>Solder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surfactant or combination of surfactants</td>
<td>8-10</td>
<td>8.6</td>
<td>10.6</td>
<td>8.6</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Sodium diethylenediamine surfactant</td>
<td>8.3</td>
<td>8.3</td>
<td>8.3</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Sodium tripolyphosphate</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Sodium pyrophosphate</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Sodium silicate</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Sodium carbonate</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Water (of hydration)</td>
<td>9.4</td>
<td>9.4</td>
<td>9.4</td>
<td>9.4</td>
<td>9.4</td>
<td>9.4</td>
</tr>
</tbody>
</table>

Example 5—Typical anionic detergent composition containing polymer-enzyme product

Additional detergent compositions are formulated according to the following specifications, using conventional procedures:

To the foregoing composition is added 0.5-5% percent by weight of each of the foregoing compositions (28 separate compositions). Combinations of these 28 polymer-enzyme products are also employed.

The compositions are dry mixed and packaged and are stable, colorless, odorless and highly enzymatically active even after long periods of storage. They are also highly active in use and extremely effective in removing ordinarily difficultly removable stains of various types from the materials cleansed therein by enzymatic action.

Particularly effective compositions are those which embody the polymer-enzyme products of the foregoing preparations, reference being made to the preparation number:

(A) 7 (K) 10+9
(B) 11 (L) 23
(C) 7+8 (M) 7+17
(D) 13 (N) 20+12
(E) 13+8 (O) 23+8
(7) 13+9 (P) 13+8+9+24
(G) 13+8+9 (Q) 9
(H) 20 (R) 8
(I) 10 (S) 13+12
(J) 10+8 (T) 18

Example 6—Typical cationic detergent composition containing polymer-enzyme product

Additional detergent compositions are formulated according to the following specifications, using conventional procedure:

To the foregoing composition is added 0.5-5% percent by weight of each of the foregoing compositions (28 separate compositions). Combinations of these 28 polymer-enzyme products are also employed.
The compositions are dry mixed and packaged and are stable, colorless, odorless, germicidally and fungicidally active, and highly enzymatically active even after long periods of storage. They are also highly active in use and extremely effective in removing ordinarily difficultly removable stains of various types from the materials cleansed therewith by enzymatic action.

Particularly effective compositions are those which embody the polymer-enzyme product of the foregoing preparations, reference being made to the preparation number:

(A) 7  (K) 10+9
(B) 11  (L) 23
(C) 7+8  (M) 7+17
(D) 13  (N) 20+12
(E) 13+8  (O) 23+8
(F) 13+9  (P) 13+8+9+24
(G) 12+8+9  (Q) 9
(H) 20  (R) 8
(I) 10  (S) 13+12
(J) 10+8  (T) 18

Example 7.—Non-ionic detergent composition containing polymer-enzyme product

Additional detergent compositions are formulated according to the following specifications, using conventional procedure:

C<sub>10</sub>-C<sub>18</sub> alcohol-ethylene oxide (5-20 moles) and/or C<sub>6</sub>-C<sub>14</sub> alkylphenol-ethylene oxide (6-20 moles) or their sulfated derivatives 5-20

Tetrasodium pyrophosphate and/or sodium tripolyphosphate 20-50

Sodium sulfate 0-25

Sodium silicate 5-20

Carboxymethyl cellulose 0.25-2.0

Optical brightener (dye) 0.02-0.5

{Polymer-enzyme product, 0.05-5 percent by weight}

To the foregoing composition is added 0.05-5 percent, preferably about 2 percent, by weight of the soluble polymer-enzyme product of each of the foregoing preparations (28 separate compositions). Combinations of these 28 polymer-enzyme products are also employed.

The compositions are dry mixed and packaged and are stable, colorless, odorless and highly enzymatically-active even after long periods of storage. They are also highly active in use and extremely effective in removing ordinarily difficultly removable stains of various types from the materials cleansed therewith by enzymatic action.

Particularly effective compositions are those which embody the polymer-enzyme products of the foregoing preparations, reference being made to the preparation number:

(A) 7  (K) 10+9
(B) 11  (L) 23
(C) 7+8  (M) 7+17
(D) 13  (N) 20+12
(E) 13+8  (O) 23+8
(F) 13+9  (P) 13+8+9+24
(G) 13+8+9  (Q) 9
(H) 20  (R) 8
(I) 10  (S) 13+12
(J) 10+8  (T) 18

For best results, the level of protease activity in a suitable detergent composition should be, for example, such that polymer-protease products are present in the detergent composition at a level of 0.05 to about 5 percent, preferably about 0.5 to about 2 percent, by weight, when the polymer-protease has an activity of about 30,000-500,000, preferably about 200,000-300,000, casein-digesting units per gram at pH 10.0 in the case of an alkaline detergent composition or such as one suitable for machine dishwashing, scouring, fabric washing, or cleansing of various types, or at a pH of about 7.0 for a hand dishwashing or other neutral detergent composition, or equivalent hemoglobin units at a pH of about 5 for a detergent composition such as may be employed in degreasing, e.g., removal of fats and greases, of metals or the like, or in cosmetic skin cleansers which may have a pH of about 5-7.

In its broader concepts, a soluble enzyme-polymer product suitable for use in accordance with the present invention is one which has its activity at a pH in the range of about 4 to about 12, preferably about 7 to about 11, and at a temperature of about 10° C. to about 85° C., preferably about 20° C. to about 76° C. Obviously, the product will be enzymatically active in the pH range produced upon employment of the detergent composition in which embodied.

The process for preparing polymer-enzyme detergent compositions of the invention comprises simply admixing the soluble polymer-enzyme product, usually in finely-divided solid form, and which is enzymatically active at a pH produced in use by the detergent or detergents with which combined, usually a pH of about 4 to about 12, preferably about 7 to about 11, a surfactant, i.e., a detergent, and any desired additional ingredients, thus to provide the novel detergent composition of the invention.

The particular polymer-enzyme product selected for employment in accord with the present invention will obviously depend on the ultimate desired end condition of use including pH, desired solubility of the product, temperatures, and type of soil, spot, or stain which is to be degraded or changed. The contact between the substrate comprising said soil, spot, or stain and said polymer-enzyme product will obviously be maintained for a period of time sufficient to enable said polymer-enzyme product to exert its enzymatic activity on said substrate, whereby either removing the same or making it susceptible to removal in or by subsequent washing or rinsing. The enzymatically active material may be chosen to give optimum activity and/or stability for any given set of conditions of use.

The polymer-enzyme products as employed in the compositions of the invention are water soluble, i.e., soluble in water at least to some extent. A water-insoluble polymer-enzyme product can be produced in accord with the prior art, if desired, by crosslinking of the polymer molecule by any one of a number of standard procedures, such as by employing a diamine or polyol crosslinking agent e.g., hexamethylenediamine, either prior to, concurrently with, or subsequent to the enzyme attachment reaction. An advantage of using an insoluble polymer-enzyme product in a composition of the invention is that such compositions can be stored for even longer periods prior to use and that, during all this time, the enzyme will be protected from moisture. At or just shortly before the time of use, however, any such insoluble polymer-enzyme product should be converted to water-soluble form by conversion of groups in the polymer molecule to produce a salt, as by the addition of base or a solvent containing a base. Another way of rendering the insoluble polymer-enzyme product water-soluble is to use the product in a strongly alkaline solution having a pH of about 8 to about 12 as is effected by some detergents, but use at such alkaline pH ranges can be made effectively only when an alkaline-acting enzyme, e.g., an alkaline protease, is incorporated into the polymer-enzyme molecule. At any rate, if an insoluble polymer-enzyme product is incorporated into the detergent composition, it should be convertible to a water-soluble form under conditions of use, as just described, which as indicated may include minor adjustments in the compositions shortly before use.

Generally, the activity of the enzymatically active polymer-enzyme product per gram is appreciable and is diluted before inclusion in end products such as detergents or presoaks. The amount of enzymatically active polymer-enzyme product incorporated into the ultimate detergent end product will depend on the amount of activity desired.
in the end product. The activity of the enzymatically active polymer-enzyme product can be diluted to any desired level with any of the following illustrative ingredients: sodium sulfate, soda ash, starch, asbestos, calcium sulfate, diatomaceous earth, silica, powdered talc, kaoline clay, or the like.

When the polymer-enzyme product is admixed with a formulated detergent, it is generally desirable to select a diluent that has about the same bulk density as that of the detergent formulation. The above-mentioned ingredients have a wide density range from about 0.1 to about 1.3 grams per ml. can be used to give the enzymatically active polymer-enzyme product a bulk density of about 0.1 to about 1.4 grams per ml., the general bulk densities of current commercial detergents. For example, soda ash, bulk density 0.35 gram per ml., can be used to dilute the enzyme activity, and the diluted polymer-enzyme product then incorporated into a bulk density detergent of about 0.3 to about 0.45 gram per ml. As a further example, soda ash and/or silica can be used to dilute the enzyme activity and the diluted polymer-enzyme product admitted with a light bulk density detergent having a bulk density of about 0.3 to about 0.5 gram per ml. From these examples, it will readily be seen that any desired activity and/or bulk density of the enzymatically active polymer-enzyme product can be formulated, and then incorporated into novel compositions of this invention.

When calcium salts, such as calcium sulfate, are used as a diluent, they appear to have the additional property of enzyme-stabilization. Detergent ingredients can also be used to dilute the activity of the polymer-enzyme product and include: trialkali metal phosphates such as trisodium phosphate; tri-potassium phosphate; dialkali metal hydroxynitrate such as disodium hydrogen phosphate and dipotassium hydrogen phosphate; alkali metal pyrophosphates, for example, tetrasodium pyrophosphate, tetrabutylammonium pyrophosphate, tetrapotassium pyrophosphate; alkali metal tripolyphosphates; and alkali metal metaphosphates such as sodium hexametaphosphate. The following may also be used: aminopolycarboxylic acids and salts such as sodium, potassium, and ammonium salts of nitrilotriacetic acid, the sodium, potassium, and ammonium salts of aminotri(methylene phosphonic acid), as well as the free acids and the diphosphonic acids and salts, methylene diphosphonic acid and 1-hydroxy-1,1-ethylenediphosphonic acid. The above materials are generally considered detergent builders, i.e., materials that enhance the detergency of the surfactant.

One or two combinations of the above-mentioned builders can be used to dilute the enzyme activity to give bulk densities that about match that of the commonly used detergents, for example, 90% granular sodium tripolyphosphate and 10% enzymatically active polymer-enzyme product are admixed and 5% of this diluted polymer-enzyme product can be further admixed with a light bulk density detergent formulation having a bulk density of about 0.3 to about 0.5 grams/ml.

When it is not desirable to decrease the amount of builder in a detergent formulation, or when one of the above-mentioned builders will not give the proper bulk density, the enzyme-polymer product can be diluted with one of the above-mentioned inert ingredients, and then combined into the detergent corporation.

A presoap product can be prepared by incorporating an enzyme-polymer and builder mixture. Illustrative of the builders are the alkali metal polyphosphates such as sodium tripolyphosphate or tetrasodium pyrophosphate, sodium silicate, sodium sulfate, or the like. However, it is more advantageous to add some surfactant to the above-mentioned mixture, such as a nonionic including the alcohol ethoxylates, or an anionic including linear alkylbenzene sulfonates. A typical presoap enzyme product contains about 85% by weight of a detergent builder such as sodium tripolyphosphate or its equivalent, about 7-11% by weight of C12 through C14 primary alcohol condensated with about 5 moles of ethylene oxide, and about 5-15% of an enzymatically active polymer-enzyme product. Such compositions are also effective even through the effective amount of polymer-enzyme product is reduced to below 1% by weight, e.g., 0.2-1%, respectively 0.5% by weight.

Additional detergent ingredients that can be incorporated into the novel compositions of the invention include surfactants such as those produced by saponification of a fatty acid such as palmitic, oleic, and the like, and the synthetic organic surfactants including the anionic, cationic, nonionic and amphoteric types and mixtures thereof.

Anionic synthetic surface active agents are generally described as those which contain hydrophilic and lyophile groups in their molecular structure and ionize in an aqueous medium to give anions containing both the lyophile groups and hydrophilic groups. The alkyl aryl sulfonates, such as sodium dodecylbenzene sulfonate; the amines salts, such as sodium dodecyl sulfate; and the sulfated oxethyethylated phenols, such as sodium tetradecyl phenoxacylxyloxy sulfates, are illustrative of the well-known class of anionic type of surface active compounds.

Nonionic surface-active agents can be broadly described as compounds which do not ionize, but which acquire hydrophilic characteristics from an oxygenated side chain such as a polyoxyethylene chain and lyophile characteristics from fatty acids, phenols, alcohols, amides, or amines. The compounds are usually made by reacting an alkylene oxide such as ethylene oxide, butylene oxide, propylene oxide, or the like with fatty acids, straight or branched chain alcohols, phenols, thiophenols, amides, and amines to form polyoxyalkylene glycol ethers and esters, polyoxyalkylene alkyl phenols and polyoxyalkylene thiophenols, polyoxyalkylene amides, and the like. It is generally preferred to react about 1 to about 30 moles of alkylene oxide per mole of fatty acid, alcohol, phenol, thiophenol, amide, or amine. Illustrative surface active agents include the product obtained from condensing ethylene oxide with the following: propylene glycol, ethylene diamine, diethylene glycol, dodecyl phenol, nonyl phenol, and the like.

Amphoteric (or amphotropic) surface active agents can be broadly described as compounds which have both an anionic and cationic group in their structure. Illustrative of amphoteric surface active agents are the amido alkane sulfonates, such as sodium C-tridecyl, N-methyl, amido ethyl sulfonate, and the like.

Other individual compounds which are illustrative of the foregoing classes of surface active agents are well-known in the art and can be found in standard detergent reference materials such as Surface Active Agents, Swartz and Perry, by Intercience Publishers, Inc., New York, N.Y. (1949).

Additional detergent ingredients that can be combined with the enzyme-polymer product and which are highly advantageous are bacteriostats such as 3,4,4-trichlorocarbinalide and the like. A perborate bleach can also be admixed with the enzymatically active polymer-enzyme product. Either of the above mentioned ingredients can be admixed with the polymer-enzyme and then admixed with a detergent or they can be admixed with a detergent that includes perborate bleaches or bacteriostats and then combined with the polymer-enzyme.

Other additional detergent ingredients that are usually found in detergent formulations can be included, such as antiredeposition agents such as carboxymethylcellulose, optical brightening agents, corrosion inhibitors, and hydro-tropes such as benzene, toluene, or xylene sulfonates to improve detergent solubility, perfumes, inert fillers, blueing agents and the like.

It will readily be appreciated that both stability and activity of the polymer-enzyme product is maintained with
little or no loss of activity when the polymer-enzyme product is combined with any of the aforementioned ingredients, despite the fact that, because of their alkalinity or acidity, they are generally incompatible with enzymes. Due to the fact that such a wide variety of ingredients can be combined with the enzyme-polymer product, a great variety of combinations having different densities and formulations can be prepared.

The soluble solid enzymatically active polymer-enzyme materials, preferably including a selected polymer-alkaline protease, can readily be comminuted to pass through the standard U.S. 20 mesh screen, although larger particles can be produced if desired. Finely-divided solid polymer-enzyme materials can be produced which are sufficiently fine to pass the standard U.S. 100 mesh screen. Generally, the greater part of the particles will be retained on a U.S. 400 mesh screen. Thus, the finely-divided solid enzymatically active polymer-enzyme materials used herein are generally within the range of from about 1 micron up to 100 microns and more commonly about 10 microns to about 100 microns.

Generally, the particle size of the polymer-enzyme product as used in the compositions of this invention ranges between about —10 (U.S. screen) and about +200 (U.S. screen) meaning that about 99% is retained on a No. 200 mesh screen. A preferred range is about —14 to +100 (U.S. screen), the particle size of commercially available detergent.

In order to color the enzymatically active polymer-enzyme product, when desired, dyes and pigments which are soluble or dispersible in water, in that it is water-soluble to the extent that at least 0.1 gram of material will dissolve in 100 grams of water at 25°C or to the extent that a dispersion or suspension of at least 0.5 gram of the coloring material can be made in 100 ml of water at 25°C, are used. Illustrative examples of coloring materials include Rhodamine B, Maxilon Pink, Auramine, Crystal Violet, Safranine, Methylene Blue, Polaris Blue, Ultramarine Blue, Sky Blue, Polar Yellow, Acridine Orange, and Aurora Pink. Colored polymer-enzyme can be admixed with detergent formulations to give a speckled appearance. Fluorescent colors can also be used to enhance the color of the dyes and pigments, such as Sodium Fluorescein. However, such coloring is not necessary, as the polymer-enzyme products used in the invention are generally colorless and odorless.

In accord with this invention a detergent formulation comprising polymer-enzyme product, an organic detergent active, and a builder can be prepared. As noted hereinbefore, by using an ordinary detergent, that is, one that is commercially available, it is difficult to remove soil or stain of a protein, hydrocarbon or fat nature such as blood, eggs, gravy, and the like. However, by using a detergent formulation containing one of the polymer-enzyme products hereinbefore mentioned, these soils can readily be removed. The amount of active polymer-enzyme that must be incorporated into a detergent to remove the above-mentioned soil generally is in the range from about 0.05% to about 5% by weight and generally from about 0.1% to about 2%, usually not greater than about 1% by weight of the detergent composition.

Any of the organic actives as described hereinbefore can be used in the detergent formulations. A particularly good class of organic surfactants, the nonionics, are condensation products of a hydrophobic compound having at least 1 active hydrogen atom and a lower alkylene oxide, for example, the condensation product of an aliphatic alcohol containing from about 8 to about 18 carbon atoms and from about 1 to about 30 moles of ethylene oxide per mole of alcohol, or the condensation product of an allyl phenol containing from about 8 to about 18 carbon atoms in the allyl group and from about 1 to about 30 moles of ethylene oxide per mole of allyl phenol. Other advantageous nonionic detergents include condensation products of ethylene oxide with a hydrophobic compound formed by condensing propylene oxide with propylene glycol. Any of the aforementioned builders can be used in the detergent formulations. Particularly good builders are, for example, sodium tripolyphosphate, potassium tripolyphosphate, trisodium nitritrocitrate, and 1-hydroxy-1-ethylidene diphosphonic acid.

The detergent formulations which incorporate the novel polymer-enzyme material of the present invention may contain any of the usual ingredients, diluents and additives, for example, perfumes, antirustine agents, antideposition agents, bacteriostatic agents, dyes, fluorescent agents, suds builders, suds depressors, foam stabilizers such as fatty alkylamides, and the like.

In preparing detergent formulations containing a polymer-enzyme product, the enzymatically active material can be directly admixed in the desired amount into the detergent formulation. Another method used in the production of a spray dried detergent is to divert a portion of the material that has already been spray dried and combine this material with the desired amount of polymer-enzyme and then reblend the mixture of polymer-enzyme and spray dried detergent with the main stream of the finished spray dried detergent particles.

In order to illustrate the invention further, the following detergent composition is prepared by directly admixing the polymer-enzyme product with an existing detergent composition.

Example 8

<table>
<thead>
<tr>
<th>Soluble Polymer-Enzyme</th>
<th>Parts by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis enzyme product (Preparation 7); diluted</td>
<td>13</td>
</tr>
<tr>
<td>Sodium alkyl benzene sulfonate</td>
<td>15</td>
</tr>
<tr>
<td>Sodium tripolyphosphate</td>
<td>41</td>
</tr>
<tr>
<td>Sodium silicate</td>
<td>6</td>
</tr>
<tr>
<td>Sodium sulfate</td>
<td>26.5</td>
</tr>
<tr>
<td>Sodium carboxymethylcellulose</td>
<td>0.5</td>
</tr>
<tr>
<td>Water (of hydration)</td>
<td>8</td>
</tr>
<tr>
<td>10% sodium tripolyphosphate granules, 10% protease, 100,000 u/gm. neutral 10% protease, 250,000 u/gm. alkaline.</td>
<td></td>
</tr>
</tbody>
</table>

Blood and grasy stains are readily removed with this composition, especially when compared with the same composition containing no polymer-enzyme product. Butter and cream stains are more readily removed when the composition embodies the polymer-enzyme products of Preparations 8, 17 and 11.

In addition to the foregoing compositions, the active polymer-enzyme products employed in accord with the present invention can be employed as a component of ordinary bar or toilet soaps, in the usual amounts ranging from 0.05 to 5% by weight, and in combination with the now well-established germicidal soaps containing hexachlorophene or the like, in the same concentrations. Ordinarily a weight concentration of less than 2% by weight, usually no greater than 1% by weight, is required, dependent on course, upon the exact use and duty for which the soap is ultimately intended.

A suitable bar soap formulation is as follows:

Example 9 —Bar soap containing polymer-enzyme product

<table>
<thead>
<tr>
<th>Percent by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium tallow fatty acid salts</td>
</tr>
<tr>
<td>Sorbitan, coconut fatty acid salts</td>
</tr>
<tr>
<td>Amphotolytic detergent, e.g., sodium coconut oil (“Coco”) fatty, acid acyl sarcosinate (Sarkosyl-TM) or sodium tallowoyl methyltaurate (Igepon T-TM)</td>
</tr>
<tr>
<td>Polymer enzyme product (any of A-T from Example 1)</td>
</tr>
</tbody>
</table>

Such products are prepared and found to have all of the advantageous properties enumerated for the compositions of Example 1 in storage and upon use. Superior stain removal properties are apparent upon use.
Suitable machine dishwashing detergent products have the following composition:

Example 10—Machine dishwashing detergent composition containing polymer enzyme product

<table>
<thead>
<tr>
<th>Sodium carbonate</th>
<th>0–20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium tripolyphosphate</td>
<td>40–85</td>
</tr>
<tr>
<td>Sodium silicate (SiO₂/Na₂O ratio of 2.8:1)</td>
<td>6–25</td>
</tr>
<tr>
<td>Water (of hydration)</td>
<td>5–15</td>
</tr>
</tbody>
</table>

Polymer enzyme product (any of A–T from Example 1) 0.1–5 preferably 0.5–2

The compositions are prepared, packaged, stored and used. They are again found to have all of the advantageous properties enumerated for the compositions of Example 1 in storage and upon use. Superior machine dishwashing is accomplished when employing these compositions.

Moreover, the polymer-enzyme products of the invention may be employed as stable, long-acting, odorless, colorless, superior and advantageous replacements for the enzymes or enzyme components of existing detergent compositions, laundering compositions, and laundering products, of all types and kinds, as are already well-known and established in the art, for example, those compositions disclosed in Guatemalan Patents F9356681, F9556676, and F956677, incorporation of the active ingredients employed according to the present invention producing the remarkable advantageous results already fully documented in the foregoing.

It is to be understood that the invention is not to be limited to the exact details of operation or exact compounds, compositions, or procedures shown and described, as obvious modifications and equivalents will be apparent to one skilled in the art, and the invention is therefore to be limited only by the full scope of the appended claims, including the application of the doctrine of equivalents thereto.

We claim:

1. Detergent composition consisting essentially of an enzymatically-active amount of an enzymatically-active polymer-enzyme product in which the enzyme is covalently bound, wherein the polymer is selected from the group consisting of:

- ethylene/maleic anhydride copolymer,
- styrene/maleic anhydride copolymer,
- vinyl methyl ether/maleic anhydride copolymer,
- vinyl acetate/maleic anhydride copolymer,
- divinyl-ether/maleic anhydride cyclocopolymer,
- polyamide anhydride,
- polyacrylic anhydride, and
cationic derivatives thereof,
said polymer-enzyme product being water-soluble or convertible to water-soluble under conditions of use, and an organic surface active agent, said polymer-enzyme product being enzymatically active in the pH range effected by the detergent in use.

2. Composition of claim 1, in the form of an anionic, cationic, nonionic, or ampholytic detergent composition.

3. Composition of claim 1 in the form of an all-purpose or heavy duty detergent composition or in the form of a bar soap.

4. Composition of claim 1, wherein the enzyme moiety of the polymer-enzyme product comprises an enzyme selected from the group consisting of neutral proteinase, alkaline protease, acid protease, amylase, and lipase.

5. Composition of claim 4, comprising a polymer-alkaline protease product.

6. Composition of claim 4, comprising a polymer-amylase product.

7. Composition of claim 1, wherein a plurality of enzymatically-active polymer-enzyme products is present and comprises at least one polymer-enzyme product selected from the group consisting of polymer-neutral product, polymer-alkaline protease, polymer-acid protease, polymer-amylase, and polymer-lipase.

8. Composition of claim 1, wherein a polymer-plural enzyme product is present and comprises at least one enzyme selected from the group consisting of neutral protease, alkaline protease, acid protease, amylase, and lipase.

9. Composition of claim 8, wherein the polymer-plural enzyme product comprises neutral protease and amylase.

10. Composition of claim 8, wherein the polymer-plural enzyme product comprises alkaline protease and another protease.

11. Composition of claim 10, wherein the ratio of the activity of the alkaline protease to the activity of the other protease in the polymer-plural enzyme product is not greater than about 0.25 to 1.2 to one.

12. Composition of claim 8, wherein the polymer-plural enzyme product comprises neutral protease, amylase and alkaline protease.

13. Composition of claim 1, wherein a polymer-enzyme product and a polymer-plural enzyme product are present and the enzyme moiety of each of the polymer-enzyme product and polymer-plural enzyme product contains at least one enzyme selected from the group consisting of neutral protease, alkaline protease, acid protease, amylase, and lipase.

14. Composition of claim 1 wherein the polymer of the polymer-enzyme product employed is an ethylene-maleic anhydride type polymer.

15. Composition of claim 1, wherein the polymer is selected from the group consisting of:

- (A) ethylene/maleic anhydride copolymer, styrene/maleic anhydride copolymer, vinyl methyl ether/maleic anhydride copolymer, vinyl acetate/maleic anhydride copolymer, divinyl ether/maleic anhydride cyclocopolymer, polyamide anhydride, polyacrylic anhydride, and cationic derivatives thereof,

and wherein the enzyme moiety comprises at least one enzyme selected from the group consisting of:

- (b) neutral protease, alkaline protease, acid protease, amylase and lipase.

16. Composition of claim 1, wherein the polymer of the polymer-enzyme product employed is ethylene-maleic anhydride.

17. Composition according to claim 1, wherein all polymer-enzyme products employed contain as the enzyme moiety or moieties thereof enzymes entirely of microbiological origin.

18. Composition of claim 1 comprising a detergent builder or bulking agent, and a polymer-enzyme product in which an alkaline protease is present as an enzyme moiety thereof.

19. Method of enzymatically removing soils, spots, or stains of an enzymatically-digestible nature which comprises the step of contacting therewith an effective amount of a water-soluble polymer-enzyme product in which the enzyme is covalently bound, wherein the polymer is selected from the group consisting of:

- ethylene/maleic anhydride copolymer,
- styrene/maleic anhydride copolymer,
- vinyl methyl ether/maleic anhydride copolymer,
- vinyl acetate/maleic anhydride copolymer,
- divinyl ether/maleic anhydride cyclocopolymer,
- polyamide anhydride,
polyacrylic anhydride, and
cationic derivatives thereof,
in the presence of an organic surface active agent, said polymer-enzyme product being enzymatically active in the pH range effected by the detergent in use, and maintaining such contact for a period sufficient to enable said polymer-enzyme product to exert its enzymatic activity on the substrate comprising said soil, spot or stain.

20. Method of claim 19, wherein the polymer-enzyme product is employed in the form of an anionic, cationic,
non-ionic, or ampholytic detergent composition and is enzymatically active at the pH range effected by said detergent composition.

21. Method of claim 20, wherein the polymer-enzyme product is employed in the form of an all-purpose or heavy duty detergent composition or in the form of a bar soap.

22. Method of claim 19, wherein the polymer-enzyme product is enzymatically active in the pH range of about 4 to about 12, preferably about 7 to 11.

23. Method of claim 19, wherein a plurality of polymer-enzyme products are employed.

24. Method of claim 19, wherein a polymer-plural enzyme product is employed.

25. Process of claim 19, wherein the polymer-enzyme product comprises an enzyme moiety thereof a neutral or alkaline protease or amylase.

26. Method of claim 19, wherein the polymer in the polymer-enzyme product is an ethylene-maleic anhydride type polymer.

27. Method of claim 19, wherein the polymer in the polymer-enzyme product is ethylene-maleic anhydride.

28. Method of claim 19, wherein the polymer is selected from the group consisting of
(a) ethylene/maleic anhydride copolymer, styrene/maleic anhydride copolymer, vinyl methyl ether/maleic anhydride copolymer, vinylacetate/maleic anhydride copolymer, divinyl ether/maleic anhydride cyclocopolymer, polymaleic anhydride, polycrylic anhydride, and cationic derivatives thereof.

and wherein the enzyme moiety comprises at least one enzyme selected from the group consisting of
(b) neutral protease, alkaline protease, acid protease, amylase, and lipase.

29. Method according to claim 19, wherein all polymer-enzyme products employed contain as the enzyme moiety or moieties thereof enzymes entirely of microbial origin.

References Cited

UNITED STATES PATENTS
3,472,783 10/1969 Smillie ............ 252—89

FOREIGN PATENTS
916,931 1/1963 Great Britain ........... 195—63

OTHER REFERENCES

LEON D. ROSDOL, Primary Examiner
W. E. SCHULZ, Assistant Examiner

U.S. Cl. X.R.
134—40; 195—63, 68; 252—Digest 12
UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,634,258 Dated 11 January 1972

Inventor(s) Bernard S. Wildi et al.

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Col. 5, line 1 "anhydried"
Page 8, lines 17-18 -- anhydride --

Col. 5, line 75 "amino hydroxyl"
Page 10, line 22 -- amino, hydroxyl --

Col. 8, line 12 "constituting"
Page 15, line 11 -- constituting --

Col. 13, 2nd figure in Table 1, column headed "Amount trypsin (mg)" "600"
Page 28, same place -- 500 --

Col. 14, Table 2, in heading (line 45) "0.1 carbonate bicarbonate"
Page 29, Table 2, in heading -- 0.1 carbonate/bicarbonate --

Col. 14, Table 2, heading "K_M" -- Km --
Page 29, Table 2, same place

Col. 14, Table 2, 3rd item "(a)"
Page 29, same place
in col. headed "Enzyme MX107"

Col. 15, line 18 "dialized"
Page 31, line 2 -- dialyzed --

Col. 15, line 29 "mg.,"
Page 31, line 11 -- mg --
It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Col. 16, line 16 "5'" 
Page 32, line 32 -- -3'-- --

Col. 20, line 61 "effected" 
Page 42, line 8 -- effected --

Col. 21, line 8 "detergent" 
Page 42, line 29 -- detergent --

Col. 21, line 12 "...ml. can" 
Page 43, line 1 -- ...ml. and can --

Col. 22, line 5 "ecective" 
Page 44, line 31 -- effective --

Col. 22, line 44 "Ampohteric" 
Page 46, line 1 -- Amphoteric --

Col. 23, line 4 "intctivate" 
Page 47, line 1 -- inactivate --

Col. 24, line 40, Ex.8 "100,000 μ/gm neutral. 10% protease,..." 
Page 50, lines 12-13 --100,000 μ/gm neutral protease --

Col. 24, line 66 "fatty, acid" 
Page 51, line 7 -- fatty acid --

Col. 25, line 45 (Claim 1) "/maleie anhydride,..." 
See amended Claim 1, line -- /maleic anhydride,... --
5 (Amendment of 2/24/71)

Col. 27, line 30 (Claim 28) "thereof." 
Claim 28, line 9 -- thereof, --

Signed and sealed this 22nd day of August 1972.

(SEAL)
Attest:

EDWARD M. FLETCHER, JR. ROBERT GOTTSCHALK
Attesting Officer Commissioner of Patents