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HIGHLY ALKALINE DETERGENT COMPOSITION CONTAINING AN ENZYME DERIVED FROM THERMOPHILIC *STREPTOMYCES RECTUS* VAR. *PROTEOLYTICUS*

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ABSTRACT OF THE DISCLOSURE

Highly alkaline enzyme-containing detergent composition of matter having a pH in the range of 9.5 to 11 and containing an organic synthetic detergent and an alkaline builder in a weight ratio of 10:1 to 1:30, and from .0025% to 10% by weight of the composition of a proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067. The detergent composition is useful as a laundering composition, a soaking composition and other cleaning applications in which it is desired to have the benefit of enzymatic activity.

CROSS REFERENCE TO RELATED CASES

This application is a continuation-in-part application of copending application Ser. No. 777,484, filed Nov. 20, 1968 now abandoned.

BACKGROUND OF THE INVENTION

This invention relates to a highly alkaline enzyme-containing detergent composition useful as a laundering or soaking composition containing an organic synthetic detergent, an alkaline builder, and an enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067.

The use of enzymes in detergent compositions is known as shown, for example, by U.S. Pat. 1,882,279 granted Oct. 11, 1932, U.S. Pat. 3,451,935 granted June 24, 1969, British Pat. 814,772 published June 10, 1959, and East German Pat. 14,296 published Jan. 6, 1958. In addition, an article in Seifen, Ole Fette, Wachse 88, No. 24, pp. 789–793 (November 1962) by Jaag discloses detergent compositions containing enzymes.

Enzymes, which are organic catalysts produced by living cells, are added to detergent compositions to aid and augment the role of the detergent in the job of removing soil and stains. Being biologically active catalysts, the enzymes degrade or break down soils and stains, for example, proteins to water-soluble peptides which the detergent can then more easily remove. By attacking and degrading the proteinaceous stains and soils in this way, the stains and soils are not available to act as binding agents for other soils and the overall cleaning and washing process is thereby materially enhanced.

In contrast to the way enzymes function, a detergent attaches its hydrophobic portion to soil particles while its hydrophilic portion orients toward the water phase. It is in this way that a detergent aids in removing ordinary soil and dirt particles during a washing process. This detergent mechanism is less effective in removing stains such as blood, milk, food residues and other protein, carbohydrate, lipid stains and the like. For this reason, it is an object of this invention to provide a detergent composition that provides the advantages of both ordinary detergents and enzymatic activity.

Enzymes are selective and catalytically effective on different types of stains and soils. In the same way that proteolytic enzymes are effective on protein stains, other enzymes degrade carbohydrate (amylases) and lipids (lipases).

While the advantages of using enzymes in detergent compositions have been known for some time; it has been difficult to provide compositions which perform successfully. There are many problems encountered in formulating enzyme-containing detergent compositions. Among the factors which must be considered are the type of enzyme employed and its compatibility with detergents, builders and other ordinary ingredients which are desirable in a complete detergent composition. Most detergent compositions intended as soaking and laundering compositions are alkaline and provide pH's in washing solutions in an alkaline range. The reason for this is that it is generally believed that better cleaning is achieved in alkaline conditions. In addition, the advantages of using hot water are also widely recognized.

While high alkalinity and hot water temperature have been objectives of detergent formulators, it is also true that these conditions are too severe for most enzymes. Enzymes are known to be highly sensitive to pH, high temperatures and water. Thus, the problem has persisted of formulating a detergent composition which would provide the advantages of high pH and at the same time provide the advantages of enzymatic activity. Not only must these conditions be taken into consideration in a washing situation, but also the enzyme can be deactivated while packaged in a detergent carton upon prolonged storage. Humidity, high pH, and high temperature can all operate to destroy an enzyme in a carton. This is undesirable because if the enzyme is not sufficiently stable under such conditions, the desired advantage of an enzyme-containing detergent composition will not be delivered to a consumer at the time of use.

That the general nature of the problems associated with satisfactorily incorporating an enzyme into a detergent composition are art-recognized can be seen in an article by Dr. Howard E. Worne, President, Enzymes, Inc., titled "The Role of Enzymes in Detergent Products," Detergent Age, September 1968.

When enzymes are not added to a detergent formula pH is not a problem and both bleaches and optical brighteners may be added to obtain optimum results. The addition of enzymes however necessitates remaining within a series of established physical and chemical parameters which allow the enzyme molecule to function without being inactivated by the presence of antagonistic ingredients. The enzymes available to the detergent industry are fragile protein molecules, and as a result, the activity may be rapidly destroyed when exposed to adverse conditions.

"Both pH and temperature are of prime importance in maintaining stability. In general, the most active pH range is from 6.0 to 9.5. Exposure to pH above and below this range rapidly denatures the protein molecule and activity drops off sharply with a resultant loss of hydrolytic efficiency.

"Such combinations, when dissolved at use strength should not result in a pH over 9.5–10. Improper formulations, particularly with alkaline silicates, alkaline phosphates and alkaline carbonates can result in extremely high pH values above 10.5 which are extremely destructive and which are not recommended."

Due to the many obstacles described above, the difficulty of achieving an effective enzyme-containing detergent composition can readily be appreciated.

SUMMARY OF THE INVENTION

An enzyme has now been discovered which uniquely possesses the characteristics which permit the attainment of each of the foregoing objectives. That is, an enzyme has been elaborated for the first time from thermophilic *Streptomyces rectus* variety *proteolytics* which is stable in a detergent composition having a pH in the range of 9.5 to 11. By contrast previously known enzymes are substantially deactivated at these highly alkaline ranges. The enzyme is also hydrolytically stable. It is also uniquely capable of withstanding the adverse effects of high temperature. Thus, this discovery makes possible a detergent composition which provides at the same time the optimum cleaning advantages of highly alkaline detergent compositions and the added cleaning power of an enzyme ingredient.

Whereas enzymes have been available which performed satisfactorily at pH's of 7 to about 9, these same enzymes were ineffective at higher pH's and for this reason could not be used satisfactorily in the highly alkaline compositions of the present invention.

The foregoing objectives are achieved according to the present invention by a highly alkaline detergent composition which comprises a water soluble, organic, synthetic detergent and an alkaline builder in a weight ratio of 10:1 to 1:30, preferably 5:1 to about 1:20, and from .0025% to 10%, preferably .003% to 3% of a proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC No. 21067, said composition having a pH in excess of 9.5 and preferably in the pH range of 9.3 to 11. This composition unexpectedly provides good cleaning and stain removing results under laundering and soaking conditions which have previously been known to inactivate heretofore known enzymatic components. The composition of this invention has several specific forms and embodiments as described and illustrated more fully below.

The discovery that a proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC No. 21067 is capable of maintaining its enzymatic activity in the foregoing composition notwithstanding the severe conditions involved in formulation and use of such a detergent composition is the basis of this invention. The enzyme elaborated from this organism is uniquely thermostable, pH stable, hydrolytically stable both in use and on prolonged storage when formulated according to the teachings of the present invention. The need for an enzyme which is active under high pH conditions is in part, accentuated by the fact that higher pH's generally result in better cleaning overall. Detergents and builders and especially salts of nitrilotriacetic acid perform best at higher pH's in the 9.5–11.0 range. There is now provided a detergent composition which has superior overall performance as a laundering and soaking composition. "Soaking compositions" is a term used to describe compositions which are added to water to which soiled and stained fabrics are added and soaked prior to an ordinary laundering cycle. Such a step is referred to in the art as a soaking or presoaking step. In order to be effective for such purposes, an enzymatic component must be sufficiently stable against the exposure to water, exposure to a highly alkaline aqueous system and/or exposure to heat depending on the temperature of the washing or soaking solution. According to the present invention, an enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067 is eminently well suited for such uses in contrast to previously known proteolytic enzymes, e.g. proteases derived from *Bacillus subtilis* microorganism.

A culture of the living organism, *Streptomyces rectus* var. *proteolyticus*, from which the enzyme is elaborated according to this invention, can be obtained from the permanent collection of the America Type Culture Collection, 12301 Parklawn Drive, Rockville, Md., U.S.A.

This strain has been assigned ATCC No. 21067. The appearance of this organism, its microscopic characterizations, and its cultural and physiological characteristics are described, in essential part, herein. A more complete characterization of this organism ATCC 21067 and a description of a process for elaborating the enzyme useful in this invention is found in separate copending patent applications Ser. No. 628,725 filed April 5, 1967, and Ser. No. 748,806 filed July 30, 1968. Both of these applications have been abandoned in favor of application Ser. No. 844,656, filed July 24, 1969, which is a continuation-in-part application of the earlier filed applications. These applications are incorporated herein by reference.

In describing the detergent composition to which the present invention pertains, a description is given below of the enzyme component, the organic synthetic detergent, and the alkaline builder ingredients. The term "detergent composition" in this description is used in a generic sense to encompass both a soaking, e.g., presoak, and a lightly or heavily built alkaline detergent composition in any physical form, e.g. solids and liquids. In other words, the composition described herein can be used in any application where enzymatic activity is desired to remove stains and soils. A preferred embodiment is as a soaking composition or a laundry composition. Optionally the composition can be used first as a soaking composition and the fabrics can then be washed in the same solution.

It is to be noted that by traditional formulation standards, an enzyme level of 10% is very high. Ordinarily the amount of enzyme used is less than 10% and typically less than about 3%. In practicing this invention, care should be exercised that for any specific composition, the amount of enzyme is not so proportionately large that autolysis of the enzyme becomes a factor. If excessive amounts of enzyme are used, it is possible that the enzyme will degrade itself.

ENZYME INGREDIENT

The enzyme which provides the unique advantages of this invention is a protease produced and elaborated from a thermophilic Streptomyces species organism isolated from a soil; more specifically, the strain of this organism is named thermophilic *Streptomyces rectus* var. *proteolyticus*. Shortened expressions such as "ATCC 21067" or "*S. rectus* var. *proteolyticus*" are also used herein to refer to the thermophilic *Streptomyces rectus* var. *proteolyticus* organism. As noted above, this organism was isolated by the Central Research Institute of Kikkoman Shoyu Company, Ltd., Noda-chi, Chiba-ken, Japan, and is on deposit with the American Type Culture Collection. It was awarded ATCC No. 21067.

The organism and its characteristics together with a process for producing and recovering an enzyme preparation therefrom is described in the following literature references, all incorporated herein by reference:

(1) Agr. Biol. Chem., vol. 28, No. 12, pp. 884–895, 1964, Studies on the Proteolytic Enzymes of Thermophilic Streptomyces, Part I. Purification and Some Properties, by Kiyoshi Mizusawa, Eiji Ichishima and Fumihiko Yoshida, Central Research Institute of Kikkoman Shoyu Company, Noda-chi, Chiba-ken.

(2) Agr. Biol. Chem., vol. 30, No. 1, pp. 35–41, 1966, Studies on the Proteolytic Enzymes of Thermophilic Streptomyces Part II. Identification of the Organism and Some Conditions of Protease Formation, by Kiyoshi Mizusawa, Eiji Ichishima, and Fumihiko Yoshida, Central Research Institute of Kikkoman Shoyu Company, Noda-chi, Chiba-ken.

(3) Applied Microbiology, March 1969, pp. 366–371, American Society for Microbiology, Production of Thermostable Alkaline Proteases by Thermophilic Streptomyces, Kiyoshi Mizusawa, Eiji Ichishima, and Fumihiko Yoshida, Central Research Institute of Kikkoman Shoyu Company, Ltd., Noda-chi, Chiba-ken, Japan.

In order to aid in understanding the present invention, two different expressions are used herein to distinguish between two different levels of purity of Streptomyces-derived enzyme preparations employed and referred to herein. One expression, "proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus*" denotes a substantially pure form of the enzyme. The other expression "Protease TP" is used to designate a relatively crude form of an enzyme preparation recovered from a fermentation process with the ATCC 21067 oragnism which preparation ordinarily contains from about 1% to about 75% by weight, preferably from about 3% to 50% of the active proteolytic enzyme and the balance 25% to 99% or 50% to 97% of inert organic and inorganic materials. Protease TP, i.e., the relatively crude fermentation product recovered from a fermentation process of ATCC 21067, in addition to containing the enzymatically-active protease ingredient, also contains certain hereinafter specified organic and inorganic ingredients. In using the name Protease TP to designate a "crude" or "relatively crude" product from a fermentation, the term "crude" is used in a broad context to include an enzyme preparation in any stage of purification between the original fermentation broth, on the one hand, and a substantially pure protease enzyme on the other hand. The final enzyme-containing fermentation product will vary in its composition depending upon the specific recovery process employed, the specific nutrient employed for fermentation and any additional ingredients added to achieve an enzyme having a standardized activity. For purposes of understanding this invention, Protease TP can be thought of as consisting essentially from 1% to 75% of the active protease enzyme and the balance 25% to 99% inert organic and inorganic materials. It should be understood that the pure proteolytic enzyme can be recovered from Protease TP by any of several convenient methods. In other words, Protease TP is merely an intermediate recovery product containing substantial amounts of inert ingredients.

DESCRIPTION OF THERMOPHILIC *STREPTOMYCES RECTUS* VAR. *PROTEOLYTICUS* ATCC 21067 AND PROTEASE TP

The organism is now described in essential details as well as the fermentation broth which contains the enzymatically active proteins, i.e., the proteases.

A Protease TP enzyme preparation is produced by inoculating thermophilic *S. rectus* var. *proteolyticus* ATCC 21067 in a culture medium that contains a suitable concentration of nutrient sources comprised of inorganic substances and carbon and nitrogen sources, etc. A cultivation treatment is carried out at temperatures ranging from 45 to 58° C. to produce a heat-resistant enzyme preparation having proteolytic activity (protease) in the culture medium which is then recovered and refined.

In the following table, the mycological properties of *S. rectus* var. *proteolyticus* ATCC 21067 are shown:

TABLE I

A. Morphological characteristics	*S. rectus* var. *proteolyticus*
Mycelium	Short branches form on a mono-axial basis, thereby almost completely becoming converted to spore chains. In the form of straight lines or waves, having a length in the range of 25 to 50μ, and a color of white.
Spore	Circular shape having a diameter of 0.8 to 1.2μ.
Substrate mycelium	Long inter-branches form, no side walls form, and no base pores form.
B. Cultivating characteristics:	
1. Czapek's agar culture medium	Unless specified otherwise, observations were made on the cultivation which was carried out for 3 days of 50° C.
Growth	Colorless, weak and spreads thin.
Mycelium	Small in amount, and white.
Soluble pigments	Does not produce any.
2. Glycerol-asparagine-agar culture medium:	
Growth	White to light brown.
Mycelium	White.
Soluble pigments	None.
3. Calcium malate-agar culture medium:	
Growth	Colorless, weak and spreads thin.
Mycelium	Small in amount, in the form of powder. Chalk white in color.
Soluble pigments	None.
4. Bouillon agar culture medium:	
Growth	Colorless, abundant, and shown wrinkles.
Mycelium	Small in amount, white.
Soluble pigments	None.
5. Bouillon culture medium	The culture medium is transparent, and the color of the culture medium does not change.
Growth	Colorless, and settles down to the bottom in the form of cotton.
6. Glucose-bouillon-agar culture medium:	
Growth	Colorless, and spreads thin. Weak.
Mycelium	None.
Soluble pigments	None.
7. Glycerol-bouillon-agar culture medium:	
Growth	Colorless and abundant. Tends to spread. Smooth.
Mycelium	None.
Soluble dyes	Light yellow.
8. Yeast extract-gelatine culture medium:	
Growth	From colorless to brown. Limiting.
Mycelium	Chalk white, powder.
Soluble pigments	Slight yellow shade.
9. Glucose-Bennet agar culture medium:	
Growth	Colorless, weak and spreads thin.
Mycelium	None.
Soluble pigments	None.
10. Maltose-Bennet agar culture medium:	
Growth	Yellow brown, thick and abundant. Shows coarse irregular wrinkles.
Mycelium	Abundant. Chalk white.
Soluble pigments	Lemon-color.
11. Glucose-Emerson agar culture medium:	
Growth	Colorless and spreads thin.
Mycelium	None. Later turns to white, 10 days after.
Soluble pigments	None. Later turns to light brown, 10 days after.
12. Maltose-Emerson agar culture medium:	
Growth	Colorless and tends to expand. Shows irregular fine wrinkels.
Mycelium	Small quantity and white.
Soluble pigments	Initially yellow gold and later turns into red brown.
13. Starch-agar culture medium:	
Growth	Yellow brown, abundant and smooth.
Mycelium	Chalk white. Powder, and abundant.
Soluble pigments	Light yellow.

TABLE I.—Continued

A. Morphological characteristics	*S. rectus* var. *proteolyticus*
14. Potato slices:	
Growth	No growth occurs.
Mycelium	
Color of the slices	
15. Carrot slices:	
Growth	No growth occurs.
16. Injection into gelatin:	
Growth	White. Sparse segments on the surface.
Mycelium	Small quantity. White.
Color of the culture medium	No changes occur.
Liquefaction	Completely liquefies.
17. Nutritious gelatin-injection:	
Growth	Small segments similar to cotton float.
Mycelium	None.
Color of the culture medium	No changes occur.
Liquefaction	Completely liquefies.
18. Litmus milk:	
Growth	Colorless thin films form on the surface.
Mycelium	None.
Solidification and peptization	A rapid solidification and peptization occurs.
C. Biological properties:	
1. Temperature range for the growth	37 to 58° C. optimum temperature 50° C. fatal temperature 100° C.
2. Oxygen	Commonly aerobic.
3. Nitrate salts	Does not undergo reduction.
4. Hydrogen sulfide	Does not form.
5. Melanine dye	Does form.
6. Starch	Undergoes hydrolysis.
7. Cellulose	Does not decompose.
8. Capability for the use of carbon. Determined by means of Primdam's method.	Cultivated for 5 days at 50° C.
L-arabinose	±
D-glucose	++
D-fructose	++
D-mannose	+
Sucrose	−
Dextrine	+++
Insitol	−
Control	−

NOTE. −+++=Good growth; ++=Rather good growth; +=Growth occurs; ±=Almost no growth occurs; −=No growth occurs.

Based on comparisons of various properties described above with those listed in Bergey's Manual of Determinative Bacteriology, 7th ed. (1957) and Waksman's The Actinomycetes, vol. 2 (1961), the organism was identified as a variety of *Streptomyces rectus* and was named *Streptomyces rectus* var. *proteolyticus* from a taxological standpoint.

CONDITIONS OF CULTIVATION FOR *S. RECTUS* VAR. *PROTEOLYTICUS*

It is necessary for the composition of a culture medium which is used in the cultivation of *S. rectus* var. *proteolyticus* ATCC 21067 to contain a carbon source and a nitrogen source as well as inorganic matters. The carbon source composed typically of sugars may suitably be comprised of starches, dextrins, maltose, etc., but glucose and cane sugar are found to be unsuitable. As the nitrogen sources, suitably used are peptone, casein, Soytone (the enzymic decomposition product of soybean manufactured by Difco Lab., U.S.A.), extracted (removed of oil) soybean, alkali extracts and other similar organic nitrogens.

The addition of a small amount of enzyme extract as a growth-promoting material, exhibits beneficial results. In addition, small quantities of inorganic salts, metal salts of magnesium, calcium, zinc, iron, manganese, etc. as well as a minute amount of nutrient material can be suitably added to the composition. Also if necessary, animal, vegetable, or mineral oils are added to the composition in the form of defoaming agents.

A heat-resistant Protease TP enzyme preparation can be produced with good yield by suitably combining the sugars and nitrogen sources as described in the foregoing. In the case where starches are used, and the extracted soybean-alkali extract is used as the nitrogen source, the optimum concentrations and weight ratios are as shown in the following table:

Organism—*S. rectus* var. *proteolyticus*
Starch, percent—2
Alkali extract of soybeans, percent—1

The cultivation temperature ranges from 45 to 60° C. at a pH level of 6.8 to 7.6. The cultivation treatment is carried out for 12 to 16 hours under aerobic conditions with vibration or with vibration and ventilation.

The greatest amount of the active proteolytic enzyme component of Protease TP appears to be produced after a cultivation of about 12 hours. Upon completion of the cultivation, the cells are removed and the clear culture filtrate thus obtained or the condensed liquor which is obtained by means of vacuum concentration, is given a customary protein refining treatment, thereby producing a standard sample of heat-resistant Protease TP.

The following examples demonstrate the cultivation processes that can be carried out in the manner described above to produce a Protease TP enzyme preparation from which a substantially pure proteolytic enzyme derived from thermophilic *S. rectus* var. *proteolyticus* can be separated and recovered.

PREPARATION A

An *S. rectus* var. *proteolyticus* organism ATCC 21067 was cultivated with vibration for 12 hours at 50° C. in a culture medium which was comprised of 2% of starch, 1.5% of Soytone, 0.1% of KH_2PO_4, 0.2% of K_2KPO_4, 0.2% of magnesium sulfate, 0.001% of calcium chloride, 0.001% of manganese sulfate, 0.001% of zinc sulfate, trace of ferrous sulfate and 0.2% of yeast extract, at a pH of 7.6. 1 ml. of the fermentation product thus produced was added to 40 ml. of the same culture medium, and a cultivation treatment was carried out with vibration for 16 hours at 50° C. The results of analyses that were carried out during the cultivation treatment, are shown in Table II.

TABLE II

[Enzymatic activity, O.D./1 ml. of cultivation liquor]

Cultivation	pH	Alkaline protease
Time:		
4	7.5	81
8	7.4	283
12	7.2	435
16	7.0	484

Upon completion of the cultivation treatment, the cells were removed by filtration, then 3 times the amount of alcohol was added to the filtrate, and the resulting pre-

cipitated fermentation mixture, Protease TP, was collected; 90% of the enzymatic activity was recovered (263 mg. from 40 ml.).

PREPARATION B

S. rectus var. *proteolyticus* ATCC 21067 was cultivated in the manner as described in Preparation A and 200 ml. of the product thus obtained was inoculated into 10 liters of a culture medium which was comprised of 2% of starch, 1% of deoleated soybeans (added in the form of a 5% alkali extract), 0.1% of KH_2PO_4, 0.2% of K_2HPO_4, 0.02% of magnesium sulfate, 0.2% of yeast extract and 0.5% of soybean oil and which was maintained at a pH of 7.5. Thereafter the cultivation treatment was carried out with agitation and ventilation in a 20 liter-capacity Waldorf's fermentation tank for 16 hours at 50° C. at 400 r.p.m. and at a ventilation rate of 5 liters/min.

The results of analysis conducted during the cultivation process are shown in Table III.

TABLE III

[Enzymatic activity, O.D./ml. of the culture solution]

Cultivation	pH	Alkaline protease
Time:		
4	7.4	10
8	7.2	44
12	6.8	86
16	6.6	99

Upon completion of the cultivation process, the culture liquor had turned into a pulp state. The precipitate thus produced as well as the cells were removed by a centrifugal treatment, and then the clear fermentation mixture thus produced was brought to a pH level of 5.0 to 5.5. Thereafter, the said mixture was vacuum condensed at 40 to 45° C. down to a volume of less than ½, than 3 times the amount of alcohol was added to the said concentrated liquor, and the precipitate thus produced, Protease TP, was gathered and dried, thereby recovering 85% of the said enzymatic activity.

Large scale production of Protease TP from *S. rectus* var, *proteolyticus* ATCC 21067 can be achieved by practicing the procedures described in a study published in Applied Microbiology, March 1967, pp. 366–371, volume 17, No. 3. This paper discusses the effects of medium composition, inoculum size and age, feeding, temperature, agitation, and aeration on production of Protease TP.

LARGE SCALE MATERIALS AND METHODS

Stock cultures

Stock cultures of *S. rectus* var. *proteolyticus* ATCC 21067 were maintained on Bennett's agar slants (modified starch) to which 10% soil extract was added. Slants were incubated at 50° C. for 24 hr. and then stored at 4° C. The spores from one slant were suspended in 10 ml. of sterile water before use.

Cultivation

Seed cultivation was conducted in a 20-liter, baffled, stainless-steel fermentor of the Waldorf type (Marubishi Rika Co., Ltd., Tokyo, Japan) with 12 liters of a medium composed of 2% soluble starch, 1.2% defatted soybean powder, 0.05% KH_2PO_4, 0.5% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2$), and 0.1% antiform (DISFORM CA–123, Nippon Oils & Fats Co., Ltd., Tokyo, Japan). The medium was inoculated with 30 ml. of spore suspension and cultivated for 16 hours at 50° C. at 400 rev./min. and 12 liters of air/min.

Fermentation procedures were conducted in 30-liter, baffled, stainless-steel fermentors of the Waldorf type with 20 liters of a medium of the same composition as the seed medium. To avoid caramelization, the mixture of phosphate solutions was sterilized separately in a 500 ml. Erlenmeyer flask. Other ingredients were sterilized by steam for 30 min. at 120° C. The medium was cooled, phosphates were added, and the medium was inoculated with 1 liter of 16 hr. seed culture (pH 7.3 to 7.4). The culture was agitated by a disk turbine impeller at 400 rev./min. Sterile air was sparged at the rate of 20 liters/min. Propagation was carried out for 24 to 48 hours at 50° C. at 0.6 kg./cm.^{2} gauge.

Assay

Samples (about 50 ml.) were taken from the fermentors every 4 hours and were immediately cooled in ice water. A 5 ml. amount was centrifuged. Protease activity was determined in the supernatant fluid by the method of Nomoto and Narahashi. [Nomoto, M., and Y. Narahashi. 1956. A proteolytic enzyme of *Streptomyces griseus*. I. Purification of a protease of *S. griseus*, J. Biochem. 46:653–667]; pH 8.0 and 30° C., and expressed as protease (PU)/ml. One PU was defined as the amount of enzyme which brought about an increase in Folin's color equivalent to 1 μg. of tyrosine per min., under the conditions specified.

Carboxypeptidase activity was assayed as follows: A 1 ml. reaction mixture (pH 8.0) containing 0.02 M carbobenzoxy-glycine-L-leucine, 0.05 M Veronal-HCl, 0.002 M $CoCl_2$, and 0.5 ml. of enzyme solution was incubated at 30° C. At appropriate intervals, 0.1 ml. samples were withdrawn and the increase of the ninhydrin color was measured by the method of Yemm and Cocking. [Yemm, E. W., and E. C. Cocking, 1955. The determination of amino acids with ninhydrin. Analyst 80:209–213.] The activity was expressed as μ moles of L-leucine liberated per minute (CPU) per milliliter.

Viscosity was measured at 4° C. with a rotary viscosimeter (model BM, Tokyo Keiki Seizo Co., Ltd., Tokyo, Japan). Mycelial growth was recorded as milliliters of wet-call volume per 2 ml. of culture broth after centrifugation at 3,000 rev./min. for 15 min. Oxygen absorption coefficient (Kd) was determined by the method of Yamada et al. [Yamada, K., J. Takahashi, and H. Okada. 1953. Fundamental studies on the aerobic fermentation II. Determination of an empirical formula on the efficiency of oxygen supply of fermentor. J. Agr. Chem. Soc. Japan 27:704–708] and expressed as gram molecule per minute per milliliter per atmosphere. Maximal rate of Protease TP production was computed from the slope of the exponential phase of production curve and expressed as PU per milliliter per hour.

RESULTS

Effect of medium constituents

Protease TP production is influenced significantly by the concentration of defatted soybean powder. The maximal production occurred at 1.2% concentration of 2.0% soluble starch. In the latter case, the C to N ratio of medium was about 10.5. The addition of inorganic nitrogen exerted no accelerating effect in this medium.

The optimal concentration of phosphate was found to be 0.33 to 0.44% 0.3 to 0.4% K_2HPO_4, 0.03 to 0.04% KH_2PO_4), which gave the highest protease yield of 540 PU/ml.

Inoculum size and age

The highest yield of enzyme was obtained with 10% inoculum among the inoculum sizes (1, 5, and 10%) examined. Inoculum age between 8 to 20 hr., which included ages from early log phase to post stationary phase of growth, showed almost no effect on yield and rate of production (the maximal yields, 470, 478, 491, and 464 PU/ml. on 8, 12, 16, and 20 hr. seeds, respectively).

Effect of feeding

To extend the exponential phase of protease production, 5 liters of two-fold concentration of fresh medium was supplied to 15 liters of culture broth at 8 to 16 hr. The production phase was delayed about 6 to 8 hr. by

the feeding, but the maximal yield increased by 20 to 30%.

Effect of temperature

Taking the maximum yield (480 PU/ml.) obtained in 16 hr. at 50° C. as 100%, the maximum yields at 45, 47.5, and 55° C. were 106% in 25 hr., 100% in 20 hr. and 71% in 16 hr. respectively. The lowest temperature, 40° C., showed the slowest rate of production. The maximum yields occurred at 50° C. (66.2), which was about seven times higher than that at 40° C.

Effect of agitation and aeration on the production of protease and carboxypeptidase

The highest protease production occurred at 400 rev./min., when the maximum yield (500 PU/ml.) was reached in 16 hr. Other agitation rates (300, 500, and 600 rev./min.) resulted in slower production rates and lower yields than did 400 rev./min. The production of carboxypeptidase did not coincide with that of protease. At 400 rev./min., the maximal activity was attained at 12 hr.; subsequently, a rapid decrease occurred, resulting in an almost complete disappearance at 24 hr. The most prominent production was observed at 500 rev./min. The maximal yield (7.3 CPU/ml.) was attained at 20 hr. when the protease activity came to peak, and the value was more than three times that obtained at 400 rev./min.

The production of protease has a different agitation dependence from that of carboxypeptidase. Kd values corresponding to the best production of protease (400 rev./min., 20 liters of air/min.) and carboxypeptidase (500 rev./min., 20 liters of air/min.) were found to be 8.1×10^{-6} and 10.9×10^{-6}, respectively. At 500 rev./min. and 4 liters of air/min., which displayed an almost identical Kd value to that of 400 rev./min. and 20 liters of air/min., the maximal yields were 504 PU/ml. for proteinase and 3.0 CPU/ml. for carboxypeptidase. Under this condition, the phase of protease production showed about 4 hr. delay, compared to that at 400 rev./min. and 20 liters of air/min.

The proteolytic enzyme produced by and derived from *S. rectus* var. *proteolyticus* ATCC 21067 by practicing the fermentation procedures described above, provides the unexpected advantages described above when used in admixture with the detergents and builders described below in the proportions and high pH conditions indicated. As noted, the amount of proteolytic enzyme derived from *S. rectus* var. *proteolyticus* which should be used in the detergent compositions is from .0025% to 10% by weight. This proportion is based on the use of a substantially pure proteolytic enzyme derived from *S. rectus* var. *proteolyticus* ATCC 21067.

Ordinarily, however, Protease TP prepared according to the previously described fermentation and recovery procedures contains an active proteolytic enzyme content of from 1 to about 75% by weight; a preferred range is from about 3 to about 50%. The balance of the Protease TP enzyme preparation is comprised of organic and inorganic salts mentioned above including non-enzyme proteins, sodium sulfate, calcium sulfate, sodium chloride, calcium chloride, sodium hydroxide, unused carbon source such as starch, water insolubles, magnesium sulfate, potassium phosphate, ethyl alcohol, as well as other organic compounds, carbohydrates, lipids, and color bodies. The enzyme preparation also has been found to provide some amylase activity in addition to proteolytic activity.

In practicing this invention, if the proteolytic enzyme derived from *S. rectus* var. *proteolyticus* is to be added to the detergent composition as a Protease TP enzyme preparation, it is necessary to know the proportionate amount of pure active enzyme contained in the crude Protease TP mixture recovered from the fermentation process before determining upon the desired amount of Protease TP which should be added to the detergent composition.

An important consideration in employing proteolytic enzymes in detergent compositions is their activity or capability for degrading and attacking proteinaceous soils and stains. The activity of a given enzyme composition is generally proportional to the relative amount of pure, active enzyme in the composition as well as the amount of the inert powdered materials noted above. As a general rule, pure samples of enzymes are the most active forms since activity is diluted by the presence of inerts.

CASEIN ASSAY

It is usual to express protease enzymatic activity in terms of activity units, e.g., casein assay activity units. By way of explanation, the casein assay method of determining proteolytic activity involves taking a solution of an enzyme preparation to be evaluated, and allowing it to digest by hydrolysis a solution of casein substrate at an appropriate pH and temperature. The reaction is stopped by the addition of trichloroacetic acid, the solution is filtered and the color of the filtrate containing the digested casein is developed employing Folic-Ciocalteu phenol reagent. The degree of enzymatic activity is determined by comparing the spectrophotometric response with that of solutions of varying concentrations of reagent grade tyrosine and determining the amounts of tyrosine produced. This casein assay method of determining proteolytic activity is well known and a more detailed discussion is found in B. Hagihara et al., J. Biochem. (Tokyo), 45, 185 (1958) and M. Kunitz, J. Gen. Physiol., 291 (1947).

While a pure sample of the enzyme derived from *S. rectus* var. *proteolyticus* ATCC 21067 has a protease activity value of about 8,000,000 units per gram, crude Protease TP enzyme preparations ordinarily have activity values which are somewhat less in the range of about 100,000 to 2,000,000 units per gram. Protease TP can be produced or standardized to provide activity all the way up to 8,000,000 units per gram. These numerical values are not significant in themselves but they do give a relative indication of enzymatic activity and offer direction to formulating proper compositions in practicing the present invention.

The amount of Protease TP which is added to the composition will ordinarily be greater if a Protease TP enzyme preparation is used which has a lower activity in the vicinity of only 100,000 units per gram than if a Protease TP preparation is used which has greater activity in the vicinity of 2,000,000 units per gram or higher. The activity of Protease TP employed has an important bearing on the degree of cleaning obtained by the detergent compositions of the present invention. The .0025% to 10%, by weight, range given for the proteolytic enzyme derived from *S. rectus* var. *proteolyticus* ATCC 21067 corresponds to the incorporation in a detergent composition of about 100,000 to 2,000,000 protease activity units per gram.

PREFERRED EMBODIMENT OF USING A MIXTURE OF STREPTOMYCES-DERIVED PROTEOLYTIC ENZYME AND AMYLASE

As noted above, a preferred embodiment of this invention is a detergent composition which, in addition to the organic detergent and the builder in the prescribed weight proportions, also contains a mixture of from .0025% to 10% of a proteolytic enzyme derived from thermophilic *S. rectus* var. *proteolyticus* ATCC 21067 and from .0003% to 3% amylase preferably from .0003% to 2% amylase. The basis for this being a preferred embodiment is the discovery that the excellent stability characteristics of the proteolytic enzyme derived from thermophilic *S. rectus* var. *proteolyticus* appears to have a beneficial stabilizing effect upon an amylase enzyme. For this reason, although one might not ordinarily consider adding an amylase enzyme to a highly alkaline (pH 9.5–11) detergent composition, the present invention now makes this possible. Accordingly, a highly alkaline detergent composition is

provided which not only affords the advantages of proteolytic activity under pH conditions of 9.5–11, but also provides amylase activity as well. By including amylase, even in small amounts, in conjunction with the thermophilic Streptomyces derived enzyme (Protease TP), a broader spectrum of soils and stains can be removed than when Protease TP is used alone. This is one of the reasons that a Protease TP/amylase mixture represents a preferred embodiment of this invention.

An amylase is a well-known enzymatically-active material which is specifically well suited for breaking down starch molecules into simple sugars as they attack the α-1,4-glycosidic linkages in starch. The resulting shorter chains are more easily removed with water or aqueous solutions of detergents. Alpha amylases are especially effective in this regard. Beta amylases, which attack terminal portions of starch molecules, are also useful.

Amylases can be obtained from a number of sources, such as animals, cereal grains and bacterial sources. Amylases from bacterial sources, especially Bacillus subtilis-containing sources, are preferred herein for reasons of ready availability, desirable rate of hydrolysis and resistance to detergent inactivation.

An amylase can be added to the compositions of the present invention in any convenient form, whether pure or in the form of a crude fermentation mixture. This parallels the situation with the Protease TP enzyme elaborated from ATCC 21067. As in the case of the proteolytic enzyme, the foregoing quantities for amylase content in the detergent compositions of this invention are likewise in terms of substantially pure form of an amylase. The source of an amylase can be a crude fermentation mixture which is a mixture of an active amylolytic enzyme and other organic and inorganic materials such as those ordinarily present, for example, in an enzyme preparation from a bacterial source. The amylase source can also simultaneously have protease activity. In determining how much of such an enzyme preparation should be used, the amount of active amylase present in the enzyme preparation must be considered. Because separation and purification techniques are expensive and time consuming, the present invention is ordinarily practiced by having the amylase source be as an enzyme preparation derived from a *Bacillus subtilis* microorganism. As contrasted with pure amylase enzymes, a crude bacterial fermentation mixture ordinarily contains about 1% to about 50% of an amylase component with the balance 50% to 99% being powdered materials (organic and inorganic) that are introduced during the production and separation (purification) process.

The crude fermentation mixture which serves as an amylase source, can also contain other enzymatically active proteins such as proteases and lipases. Such other active enzymes can contribute to the overall cleaning performance characteristics of the final composition and it is not necessary to separate them from the crude amylase-containing fermentation mixture.

Examples are given below of commercially available amylases both in purified form and also as crude fermentation enzyme preparations.

According to a preferred embodiment of this invention, Streptomyces-derived Protease TP is used in admixture with an amylase enzyme or an amylase-containing enzyme preparation such as those produced by *Bacillus subtilis*. The amount of amylolytic activity which a *Bacillus subtilis*-derived enzyme preparation can possess can be controlled by modifying production conditions.

While proteolytic activity by the *Bacillus subtilis*-derived enzyme preparation is not essential, it can be present also and contribute to overall cleaning performance. The proteolytic activity can be alkaline protease or neutral protease depending on production processes and conditions.

Specific examples of the amylase-containing enzyme preparations derived from *Bacillus subtilis* and useful herein are described in an article by Smith et al.; "The Complete Amino Acid Sequence of Two Types of Subtilisin, BPN' and Carlsberg," Journal of Biol Chem., volume 241, Dec. 25, 1966, at p. 5974. The Carlsberg subtilisin strain is characterized by a tyrosine to tryptophan ratio of about 13 to 1; the BPN' strain by a ratio of about 3 to 1. The reference including its full contents is hereby incorporated by reference.

Amylase enzyme preparations are sold by Wallerstein. A crude fermentation mixture produced from the BPN strain is available as well as mixtures of amylase and protease (obtained from Carlsberg strain) where an amylase enzyme preparation and a protease enzyme preparation is prepared separately and then these are blended together in predetermined proportions.

An amylase containing enzyme preparation suitable for use in this invention is sold by Pfizer under the trademark Maxatase. According to a trade bulletin entitled "Maxatase" published by Royal Netherlands Fermentation Industries, Ltd., Delft-Holland, this enzyme preparation is produced by cultivating a special strain of a spore forming *Bacillus subtilis* microorganism. Maxatase is a fine, free-flowing powder of a light natural color.

Milezyme, an enzyme preparation derived from a BPN-strain of *Bacillus subtilis*, has about 40% amylase activity and 60% protease activity. It is manufactured by Miles Chemical Company, Elkhart, Ind.

An X-ray mutated *Bacillus subtilis*-derived subtilisin constitutes another preferred source of amylase. The mutation can be effected in accordance with U.S. Pat. 3,031,380 issued Apr. 24, 1962, to Minagawa et al. by irradiation of a *Bacillus subtilis* organism with X-rays. A fermentation process employing this organism can then be employed to prepare an amylase enzyme composition. U.S. 3,031,380 describes a process whereby an enzyme composition is produced by subjecting a *Bacillus subtilis* microorganism to X-rays of an intensity corresponding substantially to 24–50 roentgens for an interval of at least half an hour, selecting from the colony thus subjected to X-rays a strain identified by cells having hairless, rough, jagged, spotted and dull white characteristics, separating said strain and placing this strain on a culture medium consisting of wheat bran and corn meal, maintaining the culture for a period of at least 42 hours while aerating the culture substantially continuously, and drying the broth. The disclosure of U.S. Pat. 3,031,380 is hereby incorporated by reference. The activity of this enzyme preparation is about 20% amylase, 20% alkaline protease and 60% neutral protease. Its particle size ranges predominantly from 0.03 mm. to 0.1 mm. It can be prepared to range in active enzyme content of from 20% to 75% with the balance being inert powders. The presence of calcium chloride in the enzyme powder increases the pH range over which this enzyme can be used. Monsanto Company manufactures an an amylase/protease enzyme preparation produced by a BPN-mutated strain of the type just described under the trademark Enzyme-AP.

As noted above, substantially pure amylase compositions can be utilized herein and these compositions include, for example, Wallerstein Bacterial α-Amylase, Lot No. 4546A, Wallerstein Company, Staten Island, N.Y.; ε-Amylase, Miles Chemical Company, Elkhart, Ind.; the α-Amylase which is an integral part of Monsanto's Enzyme AP described above; α-Amylase, Midwest Biochemical Company, Milwaukee, Wis.; Rapidase made by French Society Rapidase and Daismen SS and Biokleistase made by Daiwa Kasei, Japan. Mixtures of these materials can be employed to advantage in practicing the present invention.

As in the case of the preceding discussions about the proteolytic enzyme derived from thermophilic *S. rectus* var. *proteolyticus* ATCC 21067, amylase also varies in enzymatic activity depending upon several factors including concentration of the enzymatic composition, calcium ion concentration, upon pH as well as production and

recovery techniques. Pure amylase has an amylase activity of about 11,500,000 units per gram while commercially available amylase-containing preparations such as those mentioned above from *Bacillus subtilis* having activities of about 50,000 to about 2,000,000 amylase activity units/gram.

The activity values of the amylase of the present invention can be conveniently expressed in terms of an amylase assay method. In accordance with this method, a sample of amylase is allowed to catalyze the hydrolysis of the 1,4-α-glucosidic bonds of starch and glycogen for 5 minutes at a temperature of 37° C. and a pH of 6.0. The reaction is terminated by the addition of 3,5-dinitrosalicylic acid, the color is developed and the amount of maltose determined by spectrophotometric response and comparison with solutions of analytical grade maltose hydrate. The amylase has one activity unit for each 0.4 mg. of maltose hydrate produced during hydrolysis. This amylase activity method is well known and is described in P. Bernfeld, Adv. in Enzymol. 12, 379 (1951).

As described hereinbefore, a preferred embodiment of the enzyme-containing detergent composition of the present invention is characterized by the presence of about 0.0003 to about 3% by weight of amylase which corresponds to the incorporation of about 40 to 400,000 amylase activity units per gram of detergent composition. The amylase is employed in an amount such that the ratio of the proteolytic enzyme derived from *S. rectus* var. *proteolyticus* ATCC 21067 to amylase ranges about 30:1 to about 1:5. The proportions correspond roughly to a ratio of casein activity units to amylase activity units of about 50:1 to about 1:1.

Pure enzymes per se have molecular diameters of from about 30 angstroms to several thousand angstroms. However, the particle diameter of the protease and amylase enzyme preparations used herein are ordinarily larger due to agglomeration of individual enzyme molecules with each other or with inert powdered material or vehicles such as starch, organic clays, sodium or calcium sulfate or sodium chloride, added during enzyme production and recovery. As noted above, enzymes are frequently cultivated in solution. Powdered vehicles are added after filtration of such solution to precipitate the enzyme in fine form which is then dried. The enzyme powder preparations of this invention as described below are typically fine enough to pass through a Tyler Standard 20 mesh screen (0.85 mm.) although larger agglomerates are often found. Some particles of commercially available enzyme powders are fine enough to pass through a Tyler Standard 100 mesh screen. Generally a major amount of particles will remain on a 150 mesh screen. Thus, the powdered enzyme preparations utilized herein can range in size from about 1 mm. to 1 micron, most generally from 0.1 mm. to .01 mm. The enzymes used in the examples below have particle size distributions in these ranges.

The powdered materials can comprise inorganic alkali metal salts such as sodium sulfate, sodium chloride, potassium silicate, sodium phosphate, inorganic alkaline earth metal salts such as calcium sulfate, magnesium sulfate, magnesium phosphate, and the like; organic components such as nonenzymatic proteins, carbohydrates, organic clays, starches, lipids, color bodies, and the like. The portion of a crude fermentation mixture which exhibits enzymatic activity is dependent upon the manufacturing methods employed. This is not an especially critical aspect of this invention provided that, in any event, an effective amount of enzyme is employed to satisfy the weight proportions and usage concentrations given above.

ORGANIC SYNTHETIC DETERGENTS

An enzyme-containing detergent composition of the present invention contains from about 1% to about 50% of an organic synthetic detergent, preferably from about 8% to about 40%. Organic synthetic detergents suitable for use in the stable detergent compositions of the present invention include water-soluble anionic soap and non-soap synthetic detergents, nonionic synthetic detergents, zwitterionic synthetic detergents and ampholytic synthetic detergents. Mixtures of such detergents are also effective and can be used.

Examples of suitable detergent compounds of these classes which can be employed in accordance with the present invention are the following:

(a) Anionic water-soluble soap detergents: Examples of suitable soaps for use in this invention are the sodium, potassium, ammonium and alkanolammonium (e.g., mono-, di-, and triethanolammonium) salts of higher fatty acids (C_{10}–C_{22}). Particularly useful are the sodium and potassium salts of the mixtures of fatty acids derived from coconut oil and tallow, i.e., sodium and potassium tallow and coconut soaps.

(b) Anionic synthetic non-soap detergents: A preferred class can be broadly described as the water-soluble salts, particularly the alkali metal salts, or organic, sulfuric acid reaction products having in their molecular structure an alkyl radical containing from about 8 to about 22 carbon atoms and a radical selected from the group consisting of sulfonic acid and sulfuric acid ester radicals. (Included in the term alkyl is the alkyl portion of higher acyl radicals.) Important examples of these anionic synthetic detergents are the sodium or potassium alkyl sulfates, especially those obtained by sulfating the higher alcohols (C_8–C_{18} carbon atoms) produced by reducing the glycerides of tallow or coconut oil; sodium or potassium alkyl benzene sulfonates, in which the alkyl group can be a straight or branched chain and contains from about 9 to about 15 carbon atoms, preferably about 12–14 carbons; sodium alkyl glyceryl ether sulfonates, epsecially those ethers of the higher alcohols derived from tallow and coconut oil; sodium coconut oil fatty acid monoglyceride sulfates and sulfonates; sodium or potassium salts of sulfuric acid esters of the reaction produce of one mole of a higher fatty alcohol (e.g., tallow or coconut oil alcohols) and about 1 to 6 moles of ethylene oxide; sodium or potassium alkyl phenol ethylene oxide ether sulfates, with 1 to 10 units of ethylene oxide per molecule and wherein the alkyl radicals contain from 8 to 12 carbon atoms; the reaction product of fatty acids esterified with isethionic acid and neutralized with sodium hydroxide where, for example, the fatty acids are derived from coconut oil; sodium or potassium salts of fatty acid amide of a methyl tauride in which the fatty acids, for example, are derived from coconut oil; sodium and potassium salts of SO_3—sulfonated C_{10}–C_{24} α-olefins.

(c) Nonionic synthetic detergents: One class of nonionic detergents can be broadly defined as compounds produced by the condensation of alkylene oxide groups (hydrophilic in nature) with an organic hydrophobic compound, which may be aliphatic or alkyl aromatic in nature. The length of the hydrophilic or polyoxyalkylene radical which is condensed with any particular hydrophobic group can be readily adjusted to yield a water-soluble compound having the desired degree of balance between hydrophilic and hydrophobic elements. A second class of nonionic detergents comprises higher fatty amides. A third class of nonionic detergents has semi-polar characteristics. These three classes can be defined in further detail as follows:

(1) One class of nonionic synthetic detergent is marketed under the tradename of "Pluronic." These detergent compounds are formed by condensing ethylene oxide with a hydrophobic base formed by the condensation of propylene oxide with propylene glycol. The hydrophobic portion of the molecule which, of course, exhibits water insolubility, has a molecular weight of from about 1500 to 1800. The addition of polyoxyethylene radicals to this hydrophobic portion tends to increase the water solubility of the molecule as a whole and the

liquid character of the product is retained up to the point where the polyoxyethylene content is about 50% of the total weight of the condensation product.

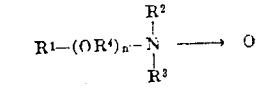
(2) Alkylphenol-polyethylene oxide condensates are condensation products of alkyl phenols having an alkyl group containing from about 6 to 12 carbon atoms in either a straight chain or branched chain configuration with ethylene oxide, the said ethylene oxide being present in amounts equal to 5 to 25 moles of ethylene oxide per mole of alkyl phenol. The alkyl substituent in such compounds may be derived from polymerized propylene, diisobutylene, octene, or nonene, for example.

(3) Nonionic synthetic detergents can be derived from the condensation of ethylene oxide with the produce resulting from the reaction of propylene oxide and ethylene diamine and include compounds containing from about 40% to about 80% polyoxyethylene by weight and having a molecular weight of from about 5,000 to about 11,000. Such compounds result from the reaction of ethylene oxide with a hypdrophobic base constituted of the reaction produce of ethylene diamine and excess propylene oxide, said base having a molecular weight of the order of 2,500 to 3,000.

(4) Other nonionic detergents include condensation products of aliphatic alcohols having from 8 to 22 carbon atoms, in either straight chain or branched chain configuration, with ethylene oxide, e.g., a coconut alcohol-ethylene oxide condensate having from 5 to 30 moles of ethylene oxide per mole of coconut alcohol.

(5) The ammonia, monoethanol and diethanol amides of fatty acids having an acyl moiety of from about 8 to about 18 carbon atoms are useful nonionic detergents. These acyl moieties are normally derived from naturally occurring glycerides, e.g., coconut oil, soybean oil and tallow, but can be derived synthetically, e.g., by the oxidation of petroleum, or by hydrogenation of carbon monoxide by the Fischer-Tropsch process.

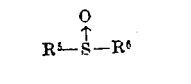
(6) Semi-polar nonionic detergents include long chain tertiary amine oxides corresponding to the following general formula



wherein R^1 is an alkyl radical of from about 8 to about 18 carbon atoms, R^2 and R^3 are each methyl, ethyl or hydroxyethyl radicals, R^4 is ethylene, and n ranges from 0 to about 10. The arrow in the formula is a conventional representation of a semi-polar bond. Specific examples of amine oxide detergents include dimethyldodecylamine oxide and bis-(2-hydroxyethyl) dodecylamine

(7) Other semi-polar nonionic detergents include long chain tertiary phosphine oxides corresponding to the following general formula RR'R''P→O wherein R is an alkyl, alkenyl or monohydroxalkyl radical containing from 10 to 20 carbon atoms and R' and R'' are each alkyl or monohydroxyalkyl groups containing from 1 to 3 carbon atoms. The arrow in the formula is a conventional representation of a semi-polar bond. Examples of suitable phosphine oxides are found in U.S. Patent 3,304,263 which issued Feb. 14, 1967, and include: dimethyldodecylphosphine oxide and dimethyl-(2-hydroxydodecyl) phosphine oxide.

(8) Still other semi-polar nonionic synthetic detergents include long chain sulfoxides having the formula



wherein R^5 is an alkyl radical containing from about 10 to about 28 carbon atoms, from 0 to about 5 ether linkages and from 0 to about 2 hydroxyl substituents, at least one moiety of R^5 being an alkyl radical containing 0 ether linkages and containing from about 10 to about 18 carbon atoms, and wherein R^6 is an alkyl radical containing from 1 to 3 carbon atoms and from one to two hydroxyl groups. Specific examples of these sulfoxides are: dodecyl methyl sulfoxides and 3-hydroxy tridecyl methyl sulfoxide.

(d) Ampholytic synthetic detergents can be broadly described as derivatives of aliphatic secondary and tertiary amines, in which the aliphatic radical can be straight chain or branched alkyls and wherein one of the aliphatic substituents contains from about 8 to 18 carbon atoms and one contains an anionic water solubilizing group, e.g., carboxy, sulfo, sulfato, phosphato, or phosphono. Examples of compounds falling within this definition are sodium-3-dodecylaminopropionate and sodium-3-dodecylaminopropane sulfonate.

(e) Zwitterionic synthetic detergents can be broadly described as derivatives of aliphatic quaternary ammonium, phosphonium and sulfonium compounds, in which the aliphatic radical can be straight chain or branched alkyl and wherein one of the aliphatic substituents contains from about 8 to 24 carbon atoms and one contains an anionic water solubilizing group, e.g., carboxy, sulfo, sulfato, phosphato or phosphono. Examples of compounds falling within this definition are 3-(N,N-diethyl-N-hexadecylammonio) propane-1-sulfonate and 3-(N,N-dimethyl-N-hexadecylammonio)-2-hydroxy propane-1-sulfonate which are preferred for their cool water detergency characteristics. See, for example, Snoddy et al., Canadian Patent 708,148.

Preferred organic detergents include sodium alkyl benzene sulfonate, sodium alkyl sulfate, and mixtures thereof wherein the alkyl group is of branched or straight chain configuration and contains about 10 to about 18 carbon atoms. Specific examples of preferred organic detergents include sodium decyl benzene sulfonate, sodium dodecyl benzene sulfonate, sodium tridecyl benzene sulfonate, sodium tetradecyl benzene sulfonate, sodium hexadecyl benzene sulfonate, sodium octadecyl sulfate and sodium tetradecyl sulfate.

These water-soluble soap and non-soap anionic, nonionic, amphlytic and zwitterionic detergent compounds can be used alone or as mixtures. The above examples are merely illustrations of the numerous suitable detergents. Other water-soluble organic detergent compounds can also be used.

ALKALINE BUILDERS

Alkaline detergency builders can be employed in a detergent composition of the present invention in a weight ratio of organic detergent to alkaline builder of about 10:1 to about 1:30, preferably 5:1 to 1:20. The detergent composition can contain from about 50% to 99%, preferably 60 to 92% of an alkaline builder ingredient. The builder can be a single ingredient or a mixture as hereinafter described.

The builders can be inorganic or organic in nature and can be selected from a wide variety of known builder materials. Useful alkaline inorganic builders are alkali metal carbonates, phosphates, polyphosphates and silicates. Specific examples of such salts are sodium and potassium tripolyphosphates, carbonates, phosphates and hexamethphosphates.

Useful alkaline organic builders are alkali metal, ammonium and substituted ammonium polyphosphonates, polyacetates and polycarboxylates. The polyphosphonates specifically include the sodium and potassium salts of ethylene diphosphonic acid, sodium and potassium salts or ethane-1-hydroxy-1,1-diphosphonic acid and sodium and potassium salts of ethane-1,1,2-triphosphonic acid. Other examples include the water-soluble [sodium, potassium, ammonium and substituted ammonium (substituted ammonium, as used herein, includes mono-, di-, and triethanol ammonium cations)] salts of ethane-2-carboxy 1,1-diphosphonic acid, hydroxy-methanediphosphonic

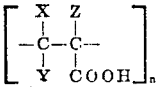
acid, carbonyldiphosphonic acid, ethane-1-hydroxy-1,1,2-triphosphonic acid, ethane-2-hydroxy-1,1,2-triphosphonic acid, propane-1,1,3,3-tetraphosphonic acid, propane-1,1,2,3-tetraphosphonic acid, and propane-1,2,2,3-tetraphosphonic acid.

Examples of the above polyphosphonic compounds are disclosed in U.S. Patents 3,159,581; 3,213,030; 3,387,024; 3,400,148; 3,400,176; 3,400,151; 3,422,021; 3,422,137.

Polyacetate builder salts useful herein include the sodium, potassium, lithium, ammonium, and substituted ammonium salts of the following acids: ethylene-diaminetriacetic acid, N-(2-hydroxyethyl)-nitrilodiacetic acid, diethylene-triamine-pentaacetic acid, 1,2-diaminocyclo- hexanetetraacetic acid and nitrilotriacetic acid. The trisodium salts of the above acids are generally and preferably utilized herein.

The polycarboxylate builder salts useful herein consist of water-soluble salts of polymeric aliphatic polycarboxylic acids selected from the group consisting of

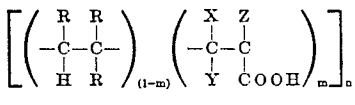
(a) Water-soluble salts of homopolymers of aliphatic polycarboxylic acids having the following empirical formula:



wherein X, Y, and Z are each selected from the group consisting of hydrogen, methyl, carboxyl, and carboxylmethyl, at least one of X, Y, and Z being selected from the group consisting of carboxyl and carboxymethyl, provided that X and Y can be carboxymethyl only when Z is selected from carboxyl and carboxymethyl, wherein only one of X, Y, and Z can be methyl, and wherein n is a whole integer having a value within a range, the lower limit of which is three and the upper limit of which is determined by the solubility characteristics in an aqueous system;

(b) Water-soluble salts of copolymers of at least two of the monomeric species having the empirical formula described in (a), and

(c) Water-soluble salts of copolymers of a member selected from the group of alkylenes and mono-carboxylic acids with the aliphatic polycarboxylic compounds described in (a), said copolymers having the general formula:



wherein R is selected from the group consisting of hydrogen, methyl, carboxyl, carboxymethyl, and carboxyethyl; wherein only one R can be methyl; wherein m is at least 45 mole percent of the copolymer; wherein X, Y, and Z are each selected from the group consisting of hydrogen, methyl, carboxyl, and carboxymethyl; at least one of X, Y, and Z being selected from the group of carboxyl and carboxymethyl provided that X and Y can be carboxymethyl only when Z is selected from the group of carboxyl and carboxymethyl, wherein only one of X, Y, and Z can be methyl and wherein n is a whole integer within a range, the lower limit of which is three and the upper limit of which is determined primarily by the solubility characteristics in an aqueous system; said polyelectrolyte builder material having a minimum molecular weight of 350 calculated as the acid form and an equivalent weight of about 50 to about 80, calculated as the acid form (e.g., polymers of itaconic acid, aconitic acid; maleic acid; mesaconic acid; fumaric acid; methylene malonic acid; and citraconic acid and copolymers with themselves and other compatible monomers such as ethylene). These polycarboxylate builder salts are more specifically described in U.S. Patent 3,308,067, issued Mar. 7, 1967 to Francis L. Diehl entitled "Polyelectrolyte Builders and Detergent Compositions."

Mixtures of any of the above-described alkaline builder salts can be utilized to advantage in this invention.

An especially preferred embodiment of this invention is one in which the builder employed is a water-soluble salt of nitrilotriacetic acid, for example, mono-, di, and trisodium nitrilotriacetate. The advantages of this preferred embodiment can be obtained in a composition which has a nitrilotriacetate builder as a sole builder. The benefits are also provided by a partial replacement of a polyphosphate builder with an equal weight basis of a nitrilotriacetate salt. In this respect, a preferred manner of practicing this invention is with a detergent composition comprising an organic synthetic detergent of the type described above, a builder mixture consisting of (a) a polyphosphate salt selected from an alkali metal polyphosphate and alkali metal pyrophosphate and (b) an alkali metal salt of nitrilotriacetate, the ratio by weight of polyphosphate to nitrilotriacetate being from 5:1 to 1.5, preferably 4:1 to 1:2, the proportion of said detergent to said builder mixture being in the range of from about 10:1 to about 1:30, by weight, from about .0025% to about 10% by weight of an enzyme derived from thermophilic Streptomyces rectus var. proteolyticus, said composition having a pH in the range of 9.5 to about 11. There can optionally be present from .0003% to 3% of amylase. Preferred cation for the builder compounds are sodium and potassium.

The basis for the nitrilotriacetate embodiment being a preferred embodiment is that this builder has its optimum building characteristics under highly alkaline conditions, i.e., 9.5–11. Consequently such a composition ideally affords the advantage of high pH detergency (saponification of lipid soils, etc.), the advantage of proteolytic and amylolytic activity, as well as the optimum benefit of a nitrilotriacetate builder. A preferred pH range for this embodiment is 9.5 to 10.8; a preferred proportion of detergent to builder mixture is 5:1 to 1:20; and, a preferred range of amylase is .0003%–2% by weight.

An alkaline detergent composition of this invention as described above and having the ingredients described herein of an organic synthetic detergent, an alkaline builder, and an enzyme elaborated from ATCC 21067 provides the superior cleaning results at a pH above 9.5 and preferably between 9.5 and 11. The composition can be used below this pH range, as other enzymes can, but the present invention is primarily based upon the surprising effectiveness and stability of the enzymatic component in a pH range which inactivates or breaks down other proteolytic enzymes. It is preferable that the composition have a pH in a range of 9.5 to 10.8. Below 9.5 cleaning performance of the composition begins to drop off especially in the embodiment in which a nitrilotriacetate builder is used. Above a pH 11, little additional benefit is obtained and this is offset by considerations of the high alkalinity on mechanical parts of the washing apparatus.

The discovery of the present invention is tied in closely to the high specific pH range. Ordinary commercial detergent compositions have and provide a pH in solution in the range of 8.5 to 9.0. Higher pH's have been avoided due to the degrading effect on previously known enzymatic components. The necessary pH range can be readily achieved by proportioning the composition in such a way that the detergent and the builder provide the necessary pH at the recommended usage levels of the detergent cohposition. It is important to note, however, that the present invention is "comprised" of a detergent component, a builder component, and the proteolytic enzyme derived from *Streptomyces rectus* var. *proteolyticus* ATCC 21067. It is possible that within the above specified proportions of detergent and builder a detergent composition may not, per se, have a pH above 9.5 as required.

In that event, the pH must be achieved or brought about either by reproportioning the detergent and builder or, alternatively, adding to the composition or the solution a pH adjuster or buffered, e.g., a suitable base such as sodium hydroxide, and the like. Ordinarily, the composition is formulated to meet the objective of having a pH in the range of 9.5 to 11. With the large number of alkaline builder materials described above, this presents no problem to those skilled in the art of formulating detergent compositions. While an upper limit of 11 has been given, it is pointed out that even higher pH's can be used. To optimize the cleaning and stability results, however, it is preferred to practice the invention within the range of 9.5 to 11.

Another consideration in formulating a detergent composition according to the directions of the present invention is the concentration of the proteolytic enzyme in the washing or soaking solution. In soaking products, the amount of enzyme tends to be higher than in products intended for ordinary household laundering applications. For effective soaking and soil removing properties, the composition should be formulated so that at ordinary usage levels there is provided a concentration of the enzyme in solution in the range of from .5 p.p.m. to about 80 p.p.m. and preferably from about 1 to about 60 p.p.m.

The temperature of the soaking or washing aqueous solution can range widely and can, in fact, be whatever temperature the consumer normally would use ranging somewhere from about 50° F. to about 200° F. The composition of the present invention is highly effective at temperatures on the order of room temperatures, e.g., 60–70° F., as well as the more usual temperatures employed in a typical household laundering in the United States, e.g., 100° F. to 160° F. Hot water temperatures traditionally involved in European soaking and laundering practices can also be used because of the aforementioned resistance to degradation of Protease TP to high temperatures, i.e., above 100° F.

The detergent compositions described herein are not restricted or limited to any special physical form. They can, for example, be solids such as granular compositions made by spray drying or coagglomeration processes or as liquid emulsion or paste (concentrates) compositions.

In addition, the detergent compositions of the present invention can also contain any of the usual detergent additives, diluents and adjuvants. For example, perfumes, anti-tarnishing agents, sodium sulfate, anti-redeposition agents such as carboxymethylcellulose, bleaches, bacteriostatic agents, dyes, optical brighteners, fluorescers, suds builders, suds depressors and the like can all be utilized herein without detracting from the advantageous stability properties of the composition of this invention.

The following compositions are illustrative of those provided and contemplated by the present invention.

EXAMPLE

A highly alkaline detergent composition embodying the present invention contains:

	Percent
Sodium dodecylbenzenesulfonate	12.5
Sodium tallow soap	2.5
Sodium tripolyphosphate	41.5
Trisodium nitrilotriacetate	9.6
Sodium silicate	10.0
Sodium sulfate	14.6
Sodium carboxymethyl cellulose	.21
Enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067	.1
Water	Balance

This composition has a pH of about 10 (which would deactivate ordinary enzymes) and provides excellent cleaning and stain-removing properties when employed in an aqueous solution in an amount that provides an enzyme concentration of 30 p.p.m. A portion of the superior cleaning results is due to the presence of the sodium nitrilotriacetate builder.

In this example, the sodium nitrilotriacetate can be a total replacement for the sodium tripolyphosphate. Similarly, the nitrilotriacetate can be replaced by an equal amount of sodium tripolyphosphate. The sodium dodecylbenzene sulfonate can be replaced with an equal weight percent of sodium tridecylbenzene sulfonate.

This highly alkaline detergent composition can effectively be used as a soaking composition as well as a usual laundering detergent composition. It has excellent stability both while being used in an aqueous solution as well as when packaged as a granular detergent.

EXAMPLE

An effective highly alkaline detergent composition incorporating the teachings of this invention contains:

	Percent
Sodium tridecylbenzenesulfonate	9.2
Sodium tripolyphosphate	59.3
Sodium silicate	5.7
Sodium sulfate	19.6
Water	6.0
Enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067	.2

This composition is effective at a pH of 9.6. It has excellent stain-removing properties and stability properties.

The enzyme-containing detergent compositions of the present invention are effective in the attainment of high levels of cleaning and soil- and stain-removal over a broad spectrum of soils and stains as demonstrated below.

STAIN REMOVING TESTS

Muslin swatches were stained by passing strips of muslin through a padding bath containing the staining solution, passing the muslin through a standard 2-roll wringer and hanging the strip to dry. Darker, more even staining was obtained by passing each strip through the staining bath to effect a second application followed by drying overnight at 120° F. The stained strip was cut into 5" x 5¼" swatches. These swatches were laundered in an automatic miniature washer at 125° F. in water of 7 grain hardness and for 10 minutes. Each composition being tested was used to wash a soiled load consisting of 3 swatches each of (1) gravy (sensitive to both proteolytic and amylolytic activity), (2) spinach (primarily measures protease activity), and (3) milk substitute (primarily measures protease activity), and (4) licorice (primarily measures amylase activity) stained muslin in the presence of two untreated terrycloth swatches added to provide bulk to the washload.

The test or control composition used for a comparison was a conventional built anionic-containing detergent formulation and was employed in an amount of 6.75 grams/1½ gal. water (equivalent to 1 cup/17 gal. water). The enzyme to be evaluated was added in the form of a fresh water solution to provide the desired level of enzyme. The swatches were washed, dried, and ironed and their whiteness levels were measured employing a Hunter Color-Difference Meter. The model employed was Model D25 from Hunter Associates Laboratory, Fairfax, Va. This device operates on the principle of reflectance and measures degree of whiteness. The greater the Hunter value the greater the whiteness level. The stain-removing effect of the enzyme was determined by comparing the Hunter Whiteness values obtained by

compositions with and without an enzyme ingredient. The control detergent for comparison purposes comprised:

Ingredient	Parts by weight
A mixture of 55% sodium tallow alkyl sulfate and 45% sodium linear alkyl benzene sulfonate wherein the alkyl chain distribution is 16% C_{11}, 27% C_{12}, 35% C_{13}, and 22% C_{14}	17.5
Sodium tripolyphosphate	50.0
Sodium silicate having an SiO_2:Na_2O ratio of 1.8:1	6.0
Coconut fatty acid ammonio amide	2.5
Sodium sulfate	14.0
Water	10.0

Results of these stain tests are tabulated in Table I. In each of the four different stains, the greater stain-removing power of the enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* is a significant one which can be recognized in an ordinary household laundering situation. As respects the gravy stain, a difference of about 2 Hunter Whiteness units is a visually observable difference; with spinach stains, an observable difference is about 4 units; with milk stains, about 1.8 units; and with licorice, about 2 units. The degrading effect of the higher pH on the activity of the subtilisin is consistently shown by comparing the stain-removing values at 10.2. The benefit obtained by the enzyme derived from the *S. rectus* var. *proteolyticus* would, according to this invention, be expected with the proteinaceous stains, i.e., gravy, spinach, and milk. However, a real advantage for the enzyme from *S. rectus* var. *proteolyticus* is evidenced even against a licorice stain which is present to note amylolytic activity.

Table IV

Enzyme derived from—	Weight percent enzyme in product [1]	Hunter whiteness: Gravy stain, pH— 9.4	Gravy stain, pH— 10.2	Spinach stain, pH— 9.4	Spinach stain, pH— 10.2	Milk stain, pH— 9.4	Milk stain, pH— 10.2	Licorice stain, pH— 9.4	Licorice stain, pH— 10.2
Bacillus subtilus derived Carlsberg strain of subtilisin [2]	0.0115	12.0	6.6	30.3	13.9	19.7	16.2	7.9	5.2
Thermophilic *Streptomyces rectus* var. *proteolyticus*, ATCC 21067	0.0115	14.5	17.1	19.1	41.1	14.6	19.9	6.7	7.3

[1] Product usage was 6.75 g/1.½ gallons water (equivalent to 1 cup/17 gallons water), providing a concentration of enzyme in solution of about 3 p.p.m.

[2] The Carlsberg strain is a known subtilisin strain, the amino acid sequence of which is described in Smith et al., "The Complete Amino Acid Sequence of Two Types of Subtilisin, BPN[1] and Carlsberg," *J. of Biol. Chem.*, Vol. 241, Dec. 25, 1966 at p. 5974. This subtilisin strain is characterized by a tyrosine to tryptophan ratio of about 13 to 1. The above reference including its description of the amino acid sequence of the Carlsberg subtilisin is hereby incorporated by reference.

The foregoing stain-removing test is a direct measurement of the ability of an enzyme to degrade stains. A complementary test was also performed to evaluate the highly alkaline enzyme-containing detergent compositions in an ordinary household laundering situation. This test confirmed that superior overall cleaning results are obtained by practicing the present invention. As a result of this testing procedure, it can be noted that the composition of this invention contribute beneficially towards the removal of the following typical stains and soils: spinach, grass, strawberries, cherries, steak sauce, chili sauce, spaghetti sauces, egg yolk, blood, baby formula (milk), tomato juice, catsup, gravy, cocoa milk, cooked vegetables, creamed corn, wine, grape juice, licorice, and the like.

The overall cleaning properties of the highly alkaline enzyme-containing compositions of the present invention were evaluated by conducting a wash-wear test described below and comparing a standardized control detergent composition to a highly alkaline enzyme-containing detergent composition of the present invention.

The wash-wear test was conducted in the following manner. White dress shirts, cotton T-shirts and other fabrics were distributed among various individuals. Each dress shirt and T-shirt was worn for one normal working day under uniform conditions and the other fabrics were used for their intended purposes. The soiled articles were then washed in a conventional automatic agitating-type washer having a water volume of 17 gallons for a 10 minute period with a control composition and an enzyme-containing detergent composition of this invention. The wash water had an average hardness of 7 grains per gallon. The control detergent composition, described previously in the description of the stain-removal test, was employed in an amount of 0.12% by weight. The enzyme was added in the form of a fresh solution in water to provide the desired level of enzyme component.

After the laundering step, the fabrics were rinsed, dried, and graded. Direct visual comparisons were made by panels of experts between pairs of shirts and fabrics worn and soiled by the same individual. The dress shirts, T-shirts and other fabrics were graded for degree of cleaning and whiteness. For purposes of this invention, the term cleaning is used to denote the ability of a built laundering detergent to remove deeply embedded soils and deposits such as occur in the collars and cuffs of white shirts. Whiteness is used as referring to an overall whiteness impression of areas which are only slightly or moderately soiled as, for example, the expansive portions of white dress shirts.

The combined data from the visual judgments were converted and were expressed on a scale ranging from zero to ten where zero represents the cleaning or whitening level obtained by washing with water alone and a value of ten represents the cleaning level of an excellent standardized detergent composition under carefully controlled optimum laboratory conditions. Table V below tabulates the results of the wash-wear evaluation. Enzyme concentration in solution was about 3 p.p.m.

TABLE V

	Cleaning grade, pH— 9.4	Cleaning grade, pH— 9.8	Whiteness grade, pH— 9.4	Whiteness grade, pH— 9.8
Control composition, no enzyme	7.0	7.0	7.0	7.0
Control detergent composition [1]	8.0	7.5	8.0	8.0
Control detergent composition [2]	8.0	[3] 9.0	8.0	[3] 8.5

[1] Containing enzyme derived from *Bacillis subtilis* (Carlsberg strain) subtilisin having 400,000 casein units/gram; .2% by weight of composition.

[2] Containing Protease TP enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067 having 650,000 casein units per gram; .2% by weight of composition.

[3] pH was 9.6.

In the results of Table II, an observable difference is one grading unit. This was obtained in the cleaning performance of the Protease TP enzyme composition. In the whiteness evaluation, a substantial directional improvement was found. By contrast the subtilisin composition cleaning directionally poorer at pH 9.8 than at 9.4; and in the whiteness evaluation the whiteness grade remained

the same, a value of 8. Even greater differences are found which reveal the improved performance of the Protease TP enzyme at more alkaline pH's. At a pH 10 for instance the degradative effect upon the subtilisin is more apparent while the Protease TP retains its enzymatic activity.

Another measurement of relative activity of an enzyme in an aqueous detergency system and of the stability of an enzyme under rigorous detergency conditions is a test called an azocollagen test. An enzyme is itself broken down as a function of time in a detergent solution with a resulting decrease in its activity of degrading and splitting proteinaceous soil and stain particles. This decrease in activity is due to the many adverse conditions all tending to degrade the enzyme molecule. These factors include all of those noted above such as hydrolytic degradation, pH, temperature, compatibility with the ingredients in the detergent composition, autolysis, and exposure to soil as well as the agitation of the washing cycle. The test to measure the relative effect of these conditions especially highly alkaline pH upon enzymes employs a common substrate for measuring proteolytic activity, i.e., azocollagen, as a function of time. The time within which one-half of the proteolytic activity is gone is termed the one-half life; the longer it takes to decrease the activity to one-half of the effectiveness, the more stable the proteolytic enzyme. The azocollagen procedure involves exposing a water-insoluble protein-dye substrate (azocollagen) to an aqueous proteolytic enzyme-containing detergent composition. The insoluble azocollagen contains a water-soluble dye which is released into the solution as a result of the enzyme hydrolyzing the azocollagen. The amount of dye released under carefully controlled conditions is measured spectrophotometrically.

In this demonstration a dyed azocollagen substrate was immersed into an aqueous solution containing .1% by weight of a composition containing:

	Percent
Sodium dodecylbenzene sulfonate	7.56
Sodium tallow alkyl sulfate	9.24
Diethanolamide	1.5
Sodium tripolyphosphate	49.4
Sodium silicate	5.9
Sodium sulfate	14.2
Sodium carboxymethylcellulose	.21
Benzotriazole	.02
Water	Balance to 100

In addition, the composition contained .0115% by weight of either a subtilisin enzyme or an enzyme derived from the ATCC 21067 Streptomyces organism. The solution had a pH of 9.65 and a temperature of 116° F. The results are in Table VI.

TABLE VI.—MEASUREMENT OF ENZYME SOLUTION STABILITY UNDER HIGHLY ALKALINE CONDITIONS

	Weight percent enzyme in product [1]	One-half life, minutes
Bacillus subtilis [2]	.0115	26
Enzyme [3]	.0115	37

[1] Concentration of enzyme in solution about 3 p.p.m.
[2] Derived (Carlsberg strain) of subtilisin having 350,000 casein activity units/g.
[3] Derived thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067 having 650,000 casein activity units/g.

It can be seen from Table VI that the Streptomyces-derived enzyme has about a 42% greater solution stability than the subtilisin enzyme under highly alkaline conditions.

An important embodiment of this invention is a process for effectively washing soiled and stained fabrics which comprises washing said fabrics with the detergent composition described herein in an aqueous solution having a pH in excess of 9.5 and generally in the range of 9.5 to 11 and a concentration of an enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067 in the range of from about 1 p.p.m. to about 80 p.p.m., preferably from 5 to about 60 p.p.m. The temperature of the aqueous solution can be in the range of 50° F. to 200° F.

Fatty acid soap detergents are mentioned above as being suitable detergents according to this invention. However, because of the particular property possessed by soaps, in that they can function in part as a detergency builder as well as a detergent (in the respect that it sequesters or ties up hardness minerals, e.g., calcium, iron, magnesium, and the like), they deserve special consideration. In addition to serving in part as builders, soaps also have a highly alkaline pH per se, in the range of 9.5 to 11. As a result, an important embodiment of this invention is a detergent composition comprising a fatty acid soap detergent containing from about 8 to about 18 carbon atoms and an effective amount of an enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067. No additional alkaline builder is needed. More especially, such a composition contains from 90 to 99.997% of said soap and from about .003% to about 10% of said enzyme and has a pH in the range of 9.5 to 11. A preferred composition useful for soaking and laundering soiled and stained fabrics comprises 97 to 99.98% of a fatty acid soap, and .02% to 3% of an enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067. A typical formulation has a pH of about 9.9.

This embodiment of the present invention is demonstrated by the following example:

	Percent
Sodium tallow soap	98
Protease TP having 10% pure proteolytic enzyme derived from ATCC 21067	2

Pure enzymes per se have molecular diameters of from about 30 angstroms to several thousand angstroms. However, the diameter of the enzyme powder preparations used herein are normally much larger due to cohesion and agglomeration of individual enzyme molecules and inert powders or vehicles added during the recovery procedure following fermentation. Examples of additives or inert materials are starch, organic clays, sodium or calcium sulfate or sodium chloride. The enzyme powders of both the Protease TP embodiment and the amylase embodiment are typically fine enough to pass through a Tyler Standard 20 mesh screen (0.85 mm.) although larger agglomerates are often found. Some particles of commercially available enzyme powders are fine enough to pass through a Tyler Standard 100 mesh screen. Generally a major amount of particles will remain on a 150 mesh screen. Thus, the powdered enzymes utilized herein can range in size from about 1 micron to 1 mm. most generally from .01 mm. to .1 mm. The enzyme powders of the examples and demonstrations described herein have a particle size in these ranges.

For purposes of practicing the present invention, the highly alkaline detergent compositions of the present invention can be prepared by methods well known to those skilled in the art. For example, the enzyme derived from thermophilic *S. rectus* var. *proteolyticus* ATCC 21067 (Protease TP) or mixture of Protease TP, amylase and/or an enzyme preparation produced from a *Bacillus subtilis* microorganism having amylase activity can be mechanically mixed into a suitably proportioned highly alkaline detergent composition in amounts specified above to provide the highly efficient and advantageous cleaning and whitening properties of the compositions described above and demonstrated below. In order to preserve the enzymatic activity of the enzyme ingredients employed, particularly under hot and humid climatic conditions, it is preferred to prepare enzyme-carrier granules which serve as a vehicle for the enzymatically active ingredients

such as Protease TP enzyme or a mixture of it with the *Bacillus subtilis*-derived enzyme preparation or other amylase source. Such enzyme-carrier granules are mixed together with detergent granules prepared by methods known in the art.

According to a preferred embodiment of this invention, an enzyme-containing detergent composition is prepared by mixing about 80% to about 98%, preferably 88% to 98% detergent granules wtih about 2% to about 20%, preferably about 2% to about 12% by weight of enzyme-carrier granules.

These preferred compositions are comprised by weight of the granular detergent composition of:

(a) about 80% to about 98% of detergent granules having a pH in aqueous solution at a concentration of about 0.12% by weight in the range of 9.5 to 11 and comprising organic detergents and alkaline builder salts, the ratio of said detergents to said builder salts being in the range of 10:1 to 1:30 by weight;

(b) About 2% to about 20% of enzyme-carrier granules having a pH in saturated aqueous solution ranging from 5.0 to 10.5, said carrier granules comprising by weight of the enzyme-carrier granules:

(1) about 20% to about 80% of phosphate builder salts selected from the group consisting of sodium tripolyphosphate and mixtures of sodium tripolyphosphate and sodium pyrophosphate;
(2) about 5% to 50% of anionic synthetic detergents of the sulfate or sulfonate type, preferably sodium alkyl benzene sulfonate, sodium alkyl sulfate, or mixtures thereof, wherein the alkyl group is of branched or straight-chain configuration and contains from about 10 to about 18 carbon atoms;
(3) about 1.0% to about 7% water; and
(4) about 0.01 to 50% of a proteolytic enzyme derived from thermophilic *Streptomyes rectus* variety *proteolyticus* ATCC 21067.

Another preferred embodiment is a composition as described above which also contains about .003% to 15% by weight of the enzyme-carrier granules of an amylase, an enzyme preparation derived from *Bacillus subtilis* having amylase activity, or a mixture of said amylase and said enzyme preparation thereof thereby providing in the composition a proportion of said proteolytic enzyme to amylase of 30:1 to 1:5.

The major portion of the preferred detergent composition of this invention, i.e. from 80% to about 98%, is comprised of detergent granules having a pH in aqueous solution at a concentration of 0.12% by weight (ordinary washing concentration) of 9.5 to 11.0. This highly alkaline pH range provides the optimum cleaning results of the present invention. These detergent granules are comprised of organic detergents and alkaline builder salts in a ratio of organic detergents to alkaline builder salts of from about 10:1 to 1:30 and preferably from about 5:1 to 1:20.

The detergent granules utilized herein are preferably formed by a spray-drying process. However, agglomerated detergent granules can be utilized with equal success. The particle sizes of the granules prepared by any process should range from about 0.1 mm. to 0.2 mm. The bulk density of the detergent granules should range from 0.2 gms./cc. to about 0.8 gms./cc. to avoid segregation in the finished detergent composition.

The remaining 2% to 20%, preferably from 2% to 12%, of the preferred granular detergent compositions of this invention is comprised of the aforementioned enzyme-carrier granules. These enzyme-carrier granules have a pH in saturated aqueous solution ranging from 5.0 to 10.5.

The enzyme-carrier granules utilized herein are especially formulated to have the same size and density characteristics as the bulk of the detergent granules to prevent segregation of the enzymes in the packaged detergent composition. This is an important consideration. Additionally, the components of the enzyme-carrier granules are so selected as to prevent lowering the efficacy of the detergent granules and of the detergent composition as a whole. The enzyme-carrier granules are also partially effective in controlling free moisture and the relative humidity in the packaged detergent composition.

The enzyme-carrier granules can be made by different methods, e.g., spray-drying or coagglomeration. The most preferred method is spray-drying. In this method, an aqueous slurry of sodium tripolyphosphate or mixtures of sodium tripolyphosphate and sodium pyrophosphate, an anionic synthetic organic detergent, e.g., sodium alkyl benzene sulfonate, sodium alkyl sulfate or mixtures thereof, is prepared. This slurry is then spray-dried to a moisture content of from about 1.0% to about 7% preferably from 1.5% to 4%. An aqueous slurry of an enzyme derived from *S. rectus* var. *proteolyticus* ATCC 21067 or a mixture of enzymes described above, e.g. an amylase-containing enzyme and Protease TP is then prepared and this aqueous slurry is sprayed onto the enzyme-carrier granules. The water in the aqueous slurry of the enzyme becomes bound as water of hydration to the enzyme-carrier granules and the enzymes are thereby attached to the enzyme-carrier granules. No more than about 7% water should be present in the enzyme-carrier granules of this invention after the aqueous enzyme slurry spray-on.

Another method of preparing the enzyme-carrier granules of this invention is by coagglomeration. In this procedure, a mixture of the various powdered detergent ingredients, i.e., sodium tripolyphosphate, an anionic synthetic organic detergent, and enzymes are sprayed with water and formed into agglomerates in a cement mixer, pan agglomerator or the like. The agglomerates so formed, i.e., enzyme-carrier granules, should be approximately the same size and density as those utilized in the bulk of the detergent composition.

Phosphate builder salts selected from the group consisting of sodium tripolyphosphate and mixtures of sodium tripolyphosphate and sodium pyrophosphate are major components of the enzyme-carrier granules and are utilized herein in amounts ranging from about 20% to about 80% by weight of the enzyme-carrier granules and preferably in amounts ranging from about 40% to about 65%. The preferred phosphate builder salt is sodium tripolyphosphate.

Mixtures of the sodium salts of tripolyphosphoric acid and pyrophosphoric acid, whether formed by heat degradation of sodium tripolyphosphate or by mixing the two phosphate builder salts, can contain up to 100% sodium tripolyphosphate and not more than about 45% sodium pyrophosphate. Preferably, the amount of sodium pyrophosphate should not exceed about 25%. It is typically present in heat-dried sodium tripolyphosphate in amounts in excess of about 5% and up to about 25%.

Sodium tripolyphosphate is a valuable component of the enzyme-carrier granules because it provides alkaline builder characteristics and bulk of these granules. This salt is especially valuable herein because in it anhydrous or partially hydrated form it acts as a moisture sink or desiccant and thus controls, to some extent, free moisture in the packaged detergent composition. Therefore, it is preferred that the sodium tripolyphosphate be utilized herein in its anhydrous or partially hydrated form.

The organic detergent used in the enzyme-carrier granules helps to prevent undesirable product dustiness (a large portion of very fine particles). The organic detergents also contribute to the overall washing capabilities of the detergent composition.

The detergent composition of the present invention in granular form can also contain a minor amount of water, i.e., up to about 15% of the detergent composition. There is ordinarily present from 1% to about 12%.

A highly efficient granular detergent composition prepared according to this invention has the following ingredients mechanically mixed together:

	Percent
Sodium linear alkyl benzene sulfonate having an alkyl chain-length distribution of 16% C_{11}, 27% C_{12}, 35% C_{13}, 22% C_{14}	20
Sodium tripolyphosphate	50
Sodium nitrilotriaacetate	20
Balance	9
Proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus*, ATCC No. 21067	.2
Amylase enzyme	.05

The composition is an especially efficient detergent composition in a washing solution having a pH 9.8 and when added in an amount that provides a concentration of the enzyme of 10 p.p.m.

Besides having excellent overall cleaning properties in a typical household laundering application, the composition is a highly effective soaking composition. The soaking step can be followed by an ordinary washing cycle in the soaking solution.

Fabrics washed with the detergent composition of this example are effectively cleaned not only in overall appearance but also in more heavily soiled areas such as collars and cuffs of shirts. In addition, the increased efficacy of the proteolytic enzyme in breaking down and removing proteinaceous soils is readily apparent from examination of strained areas on the laundered fabrics. The amylase enzyme can be replaced with an equal weight amount of an enzyme preparation derived from *Bacillus subtilis* having amylolytic activity, such as Milezyme or Maxatase, as defined above.

The following composition is an excellent granular detergent composition useful for laundering soiled and stained fabric including soaking applications. It can be prepared by mixing the ingredients together.

	Percent
Sodium alkyl benzene sulfonate, the alkyl group average 11.8 carbon atoms derived from tetrapropylene	8
Sodium tallow alkyl sulfate	10
Sodium tripolyphosphate	40
Sodium nitrilotriacetate	10
Sodium sulfate	14
Sodium silicate (1.6 ratio)	6
Proteolytic enzyme derived from thermophilic *S. rectus* var. *proteolyticus* ATCC No. 21067	.1
Enzyme preparation derived from *Bacillus subtilis* having about 80% protease activity, about 20% amylase activity	.033
Water	Balance

When used at a concentration in a washing solution having pH 10.0, a highly efficient overall cleaning performance is obtained. The composition has a pH of 9.6.

Results substantially similar to those obtained in the previous two described compositions are obtained when the following builder salts are substituted, either wholly or in part, for sodium tripolyphosphate in that the composition is an effective laundry detergent: sodium potassium, ammonium, monoethanol ammonium, diethanol ammonium and triethanol ammonium salts of the following acids:

ethylenediaminetetraacetic acid;
N-(2-hydroxyethyl)-ethylenediaminetriacetic acid;
N-(2-hydroxyethyl)-nitrilodiacetic acid;
diethylenetriaminpentaacetic acid;
nitrilotriacetic acid;
ethylene diphosphonic acid;
ethane-1-hydroxy-1,1-diphosphonic acid;
ethane-1,1,2-triphosphonic acid;
ethane-2-carboxy-1,1-diphosphonic acid;
hydroxymethane-diphosphonic acid;
carbonyl-diphosphonic 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-tetraphosphonic acid; and
propane-1,2,2,3-tetraphosphonic acid and potassium tripolyphosphate; and salts of polymers of itaconic acid, aconitic acid, amleic acid, mesaconic acid, fumaric acid, methylene malonic acid and citraconic acid and copolymers with themselves and/or ethylene and/or acrylic acid in, e.g., 1:1 molar ratios and having molecular weight of 75,000; 100,000; and 125,000 (the copolymers with ethylene and/or acrylic acid having equivalent weights, based on the acid form of 65, 70 and 75); in the form of their sodium, potassium, triethanolammonium, diethanolammonium and monoethanolammonium salts.

Results substantially similar to those obtained in these examples are obtained when the following organic detergents are substituted, either wholly or in part, for the sodium alkyl benzene sulfonate or mixtures of sodium alkyl benzene sulfonate and sodium tallow alkyl sulfate in that the composition is an effective laundry detergent: sodium linear dodecyl benzene sulfonate, the condensation product of 1 mole of dodecyl phenol with 15 moles of ethylene oxide, dimethyldodecylamine oxide, dimethyldodecylphosphine oxide, 3-(N,N-dimethyl-N-hexadecylammonio)-2-hydroxypropane-1-sulfonate and sodium-3-dodecylaminopropane sulfonate.

The proteolytic enzyme derived from thermophilic *S. rectus* var. *proteolyticus* can be combined with an amylase-containing enzyme preparation derived from *Bacillus subtilis*. The presence of the amylase content does not interfere with the efficient cleaning properties which have now been discovered to be possessed by enzymes derived from thermophilic *S. rectus* var. *proteolyticus* in a pH range of 9.5 to 11.0. For many household cleaning and laundering applications, it is preferred to use a mixture of a proteolytic enzyme and an amylase enzyme, such as for laundering and dishwashing.

The detergent composition of the present invention besides offering the new and unexpected benefit of being so highly effective in a pH range of 9.5 to 11.0 also have other advantages. For example, the enzymatic activity of the thermophilic Streptomyces-derived enzyme is stable over a broad temperature range. The compositions described herein are more resistant to hydrolytic attack and inactivation due to highly alkaline ingredients of detergent compositions. Still further, the detergent compositions of this invention provide excellent stability under severe and varied storage conditions while the detergent is packaged in cartons.

Still further, the enzyme-containing compositions of this invention are compatible with a very large range of detergents and builders.

Besides those enzymes mentioned above, others can also be added to provide detergent compositions having even greater enzymatic activity. Thus, for instance, it is possible to optionally add pepsin, trypin, chymotrypsin, papain, bromelin, colleginase, keratinase, carboxylase, amino peptidase, elastase, subtilisin and aspergillopepidase A and B. Suitable optional enzyme ingredients commercially available include: Monzyme (Monsanto Chemical Company), Protease AP (Sandoz-Ferment, Basel, Switzerland), Protease B–400 (Sandoz-Ferment), Protease ATP 40 (Sandoz-Ferment), Pancreatin NF (Pfizer), Pancreatin 6xNF (Armour), Fungal Protease (Miles), DSE Numbers 4–9 (Rohm and Haas), Enzyme DPX (Premier Malt), Protease L–252 Digester (Premier Malt), Protease L–253 Digester (Premier Malt), Protease L–423 (Premier Malt), Protease L–516 (Premier Malt), Protease L–517 (Premier Malt), Texzyme PX–1 (Premier Malt),

Protease P-G (Pfizer), Compounds 37B (Miles), Serizyme (Wallerstein), Papin 100 (Wallerstein), Optimo Papain Penick), Ficin (Miles), Bromelain (Miles), HT Proteolytic Concentrate Miles), Protease ATP 40 (Rapidase), Protease ATP 120 (Rapidase), Rhozyme P-11 (Rohm and Haas) and Rhozyme PF (Rohm and Haas).

Metalloproteases which contain divalent ions such as calcium, magnesium or zinc bound to their protein chains can also be used.

A noteworthy advantage of the detergent compositions of the present invention is the unexpectedly superior shelf stability of such compositions when packaged. This stability appears to be related to the hydrolytic-stability explained and demonstrated above. It might seem that the small amount of moisture in a carton or container of the granular product would not have deleterious effects. As a matter of fact, it does, to the extent the previously employed proteases are very markedly destroyed or impaired. By contrast, the enzymes derived from thermophilic *Streptomyces rectus* var. *proteolyticus* 21067 are unexpectedly stable in the presence of the small pockets of moisture present in packaged detergent compositions. High pH's and excessive temperatures in packaged detergent compositions can combine to present a severe test of the shelf stability of an enzymatic component. The compositions of the present invention provide excellent results even under the most severe environmental conditions.

To demonstrate a preefrred mode of practicing the enzyme-carrier granule embodiment of this invention, the following description is provided of a highly alkaline detergent composition of the present invention.

EXAMPLE

Enzyme-carrier granules were prepared by mixing the following ingredients into a detergent slurry:

Ingredient	Parts by weight
Anionic organic detergent paste [1]	36.17
Sodium tripolyphosphate	70.03
Water	28.22
Sodium sulfate	4.55
Total	138.97

[1] The organic detergent paste containing in parts by weight: 5.06 parts sodium tallow alkyl sulfate; 4.14 parts sodium linear alkyl benzene sulfonate having an alkyl chain length distribution of 16% C_{11}, 27% C_{12}, 35% C_{13}, and 22% C_{14}; 6.16 parts sodium sulfate; and 20.81 parts water.

This slurry was mixed until it was homogeneous after which the slurry was spray-dried to a total moisture content of 3.88%. 93.39 parts of enzyme-carrier granules were thus obtained.

When this slurry was spray-dried, a portion of the sodium tripolyphosphate was degraded. The final distribution of phosphate salts on the basis of the original sodium tripolyphosphate added was 75.5% sodium tripolyphosphate; 21.4% sodium pyrophosphate and 3.1% sodium orthophosphate. These phosphate salts were partially hydrated.

Two parts of a mixture of Protease TP containing about 9% by weight active protease of a proteolytic enzyme derived from thermophilic *S. rectus* var. *proteolyticus* ATCC 21067 and balance inerts; the enzyme having an alkaline protease activity of about 650,000 casein units per gram at pH 10.3 using the casein assay method described above; 0.50 parts of an enzyme preparation called Milezyme derived from a BPN-strain of Bacillus subtilis containing about 5% by weight alkaline protease and about 6%, by weight, amylase, the protease having activity about 450,000 casein units per gram and the amylase having activity of about 700,000 amylase units per gram; and 2.50 parts water were slurried together and sprayed onto the 95.00 parts of enzyme-carrier granules obtained above. The water was bound as water of hydration by the sodium tripolyphosphate and the enzymes were attached to the enzyme-carrier granules. The pH of these enzyme-carrier granules in saturated aqueous solution was about 8.5. Five parts of these enzyme-carrier granules were mixed with 95 parts of spray-dried detergent granules which had a pH of about 9.6 in aqueous solution at a concentration of 0.12% by weight and which comprised in parts by weight of the detergent granules.

Ingredient:	Percent
Sodium tridecyl benzene sulfonate	12.6
Coconut alcohol-ethylene oxide condensation product having 6 ethylene oxide units	.5
Sodium tripolyphosphate	41.5
Sodium nitrilotriacetate	9.6
Sodium silicate (ratio of SiO_2:Na_2O of 1.8:1)	7.0
Sodium sulfate	16.0
Water	10.0
Brightener	.6
Perfume	.23
Miscellaneous	Balance

This detergent composition contains .1% Protease TP (.006% pure proteolytic enzyme derived from *S. rectus* var. *proteolytic* ATCC 21067) and .025% Milezyme (5% alkaline protease and 6% amylase enzyme). This highly alkaline detergent composition embodies several of the preferred embodiments described above including a highly alkaline pH 9.6, a nitrilotriacetate builder, and an enzyme mixture of protease and amylase. In full scale wash-wear tests, this composition performs exceedingly well in overall cleaning and whiteness results. Not only are stains removed very well, but especially good results are observed in areas heavily soiled by skin particles. One a grading scale of 1–10, this composition receives a value of 9 which indicates very satisfactory laundering results.

It has also been discovered that the proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067 is unexpectedly efficient in providing enzymatic activity in a detergent composition. In other words, in a detergent composition having a pH in the range of about 8 to about 12, a small amount of the thermophilic Streptomyces-derived proteolytic enzyme provides unexpectedly superior cleaning results over an equal amount of any other known proteolytic enzyme. It requires a substantially greater amount of any other proteolytic enzyme to attain the same level of cleaning obtained by a detergent composition which has a given amount of enzyme derived from Streptomyces ATCC 21067. This discovery is more completely described in a copending patent application filed by John Siebert, Robert L. Gensler, Kiyoshi Mizusawa, Eiji Ichishima, and Fumihiko Yoshida, entitled "Detergent Composition Containing an Enzyme Derived From Thermophilic *Streptomyces Rectus* Variety Proteolyticus." According to that invention, there is provided a detergent composition comprising

(a) An organic detergent,

(b) A detergency builder selected from the group consisting of an inorganic alkaline builder, an organic alkaline sequestering builder, and mixtures thereof, the proportion of said detergent to said builder being in the range of from about 10:1 to about 1:30, by weight, and

(c) From about .0025% to about 2% by weight, of a proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus*.

The following is an example of a composition which embodies the discovery of the increased efficiency of Protease TP:

The following composition is an excellent granular detergent composition useful for laundering soiled and stained fabrics including soaking applications. It can be prepared by mixing the ingredients together.

	Percent
Sodium alkyl benzene sulfonate, the alkyl group averaging 11.8 carbon atoms derived from tetrapropylene	8
Sodium tallow alkyl sulfate	10
Sodium tripolyphosphate	50
Sodium sulfate	20
Sodium silicate (1.6 ratio)	6
Pure proteolytic enzyme derived from thermophilic *S. rectus* var. *proteolyticus* ATCC No. 21067	.1
Pure amylase	.033
Water	Balance

When used at a concentration in a washing solution having pH 9.4 of 4 p.p.m. of total active enzyme, a highly efficient overall cleaning performance is obtained.

Still further, it has also been discovered that the proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067 is uniquely stable in a detergent composition containing an oxidizing bleaching agent such as sodium perborate. This invention is embodied in copending patent application Ser. No. 777,485, filed Nov. 20, 1968, by Everett J. Collier with the title "Detergent Composition Containing an Oxidizing Bleaching Agent and an Alkaline Proteolytic Enzyme Derived From Streptomyces." The following composition includes the embodiment of the surprising stability of Protease TP in a perborate-containing detergent composition:

	Percent
Sodium dodecylbenzenesulfonate	12.5
Sodium tallow alkyl sulfate	.4
Sodium tripolyphosphate	41.5
Trisodium nitrilotriacetate	9.6
Sodium silicate	10.0
Sodium perborate	7.0
Sodium sulfate	9.9
Enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067	.1

Both of these copending patent applications are incorporated herein by reference.

Having described the present invention in its several embodiments, what is claimed is:

1. A highly alkaline detergent composition consisting essentially of:

(I) from about .0025% to about 10% by weight of a proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus*, and

(II) the balance of said detergent composition consisting essentially of (a) an organic synthetic detergent, and (b) an alkaline builder selected from the group consisting of an organic sequestering builder, an inorganic alkaline builder and mixtures thereof, the proportion of said detergent to said builder being in the range of from about 10:1 to 1:30 by weight, and

wherein said composition has a pH in the range of 9.5 to about 11.

2. A detergent composition according to claim 1 in which the prooprtion of said detergent to said builder is in the range of from about 5:1 to about 1:20.

3. A detergent composition according to claim 1 in which said proteolytic enzyme is present in an amount of from about .003% to about 3%.

4. A detergent composition according to claim 1 which also contains from .0003% to 3% of amylase.

5. A detergent composition according to claim 4 in which the amylase is present in an amount of .0003% to 2% amylase.

6. A process for washing soiled fabrics which comprises washing said fabrics in an aqueous solution containing the detergent composition of claim 1 in an amount sufficient to provide a pH in the range of about 9.5 to about 11 and a concentration of said proteolytic enzyme in the range of from about .5 p.p.m. to about 80 p.p.m.

7. A process according to claim 6 in which the aqueous solution has a temperature in the range of from about 50° F. to about 200° F.

8. A process according to claim 7 in which the concentration of said proteolytic enzyme is in the range of from about 1 to about 60 p.p.m.

9. A detergent composition consisting essentially of

(a) from about 90 to about 99.997% fatty acid soap, and

(b) from about .003% to about 10% of a proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus*,

said composition having a pH in the range of about 9.5 to about 11.

10. A process for washing soiled fabrics which comprises immersing said fabrics in an aqueous solution containing the detergent composition of claim 8 in an amount sufficient to provide a concentration of said proteolytic enzyme in the range of from about .5 p.p.m. to about 80 p.p.m.

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