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DETERGENT COMPOSITIONS CONTAINING AN OXIDIZING BLEACH AND PROTEOLYTIC ENZYME DERIVED FROM THERMOPHILIC *STREPTOMYCES RECTUS* VAR. *PROTEOLYTICUS*

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No Drawing. Continuation-in-part of abandoned application Ser. No. 777,485, Nov. 20, 1968. This application Aug. 15, 1969, Ser. No. 850,656
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ABSTRACT OF THE DISCLOSURE

Detergent composition containing an organic synthetic detergent and an alkaline builder in a weight ratio of about 1:30 to about 5:1, an oxidizing bleach and a proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067. These compositions are useful in any application where the combined effect of an oxidizing bleach and proteolytic enzyme activity are desired, such as laundering and soaking compositions.

CROSS REFERENCE TO RELATED CASES

This application is a continuation-in-part application of copending application Ser. No. 777,485, filed Nov. 20, 1968, now abandoned.

FIELD OF THE INVENTION AND BACKGROUND

This invention relates to a detergent composition which contains an organic synthetic detergent, an alkaline builder, an oxidizing bleach and a proteolytic enzyme. Detergent compositions containing mixtures of the three former ingredients have been known for a long time, that is synthetic detergents, builders, and an oxidizing bleach such as perborate. Detergent compositions have also been known which contain enzymes to aid in the soil and strain-removing process.

In all previous known attempts to combine such embodiments into a single detergent composition, that is, one which contains a detergent, a builder, a bleach, and an enzyme, it has been necessary to protect the enzyme from degradative attack by the bleaching agent. While this has been fairly satisfactorily done, as for example, by the technique described in copending application Ser. No. 635,293, filed Apr. 12, 1967, by McCarty now U.S. Pat. 3,519,570, granted July 7, 1970, separate steps and added materials are required in the production process. In addition, such additional steps required to protect the enzyme from the bleach represent considerable added expense. Even in such embodiments, the amount of enzyme used is considerably greater than usual to compensate for any loss of enzyme due to attack by a bleach, such as sodium perborate.

The objective of preparing a superior detergent composition that provides both bleaching action from a bleach such as perborate and an enzyme to aid in removal stains and proteinaceous soils has been one of long standing.

Published literature has recognized the difficulty of meeting this objective. For example, Dr. Howard E.

Worne, Enzymes, Inc., in an article published in Detergent Age, September 1968, titled "The Role of Enzymes in Detergent Compositions" stated:

"Bleaching agents such as perborates, peroxides or compounds containing active chlorine, strongly inhibit the reaction of proteolytic enzymes, and as such, they must be eliminated from all proposed formulation."

In light of this clear statement in the prior art reflecting the state of the art concerning the incorporation of enzymes into detergent compositions containing an oxidizing bleaching agent, the contribution of the present invention can be more readily appreciated.

SUMMARY OF THE INVENTION

According to the present invention, the foregoing objective are achieved because it has now been discovered that an improved highly effective alkaline detergent composition providing both bleaching and enzyme activity consists essentially of an organic synthetic detergent and an alkaline builder salt in a weight ratio of from about 10:1 to about 1:30, preferably 5:1 to about 1:20, from about 2.5% to about 60% of an oxidizing bleach, preferably 10% to about 50%, and from about .0025% to about 10%, preferably .0025% to 3%, of a proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067.

The present invention has been made possible because an enzyme has now been discovered which uniquely possesses the characteristics which permit the attainment of the foregoing objective. That is, an enzyme has been elaborated for the first time from thermophilic *Streptomyces rectus* variety *proteolyticus* ATCC 2167 which is stable in a detergent composition having an oxidizing bleach in admixture with a synthetic detergent and an alkaline builder. By contrast previously known enzymes are substantially deactivated by an oxidizing bleaching agent unless the specific precautionary steps are taken to protect the enzyme. As an added advantage, 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 can provide at the same time the desirable cleaning advantages of highly alkaline detergent compositions containing an oxidizing bleach, and the added cleaning power of an enzyme ingredient.

The discovery that a proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC No. 21067 is capable of maintaining enzymatic activity in the foregoing composition notwithstanding the exposure to an antagonistic ingredient such as an oxidizing bleach is the basis of this invention. The enzyme elaborated from this organism is, in addition to being bleach-stable, uniquely thermostable, pH stable, and 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 these conditions is in part, accentuated by the fact that these conditions are almost always present in an ordinary household washing situation. Detergents and builders and especially salts of nitrilotriacetic acid perform best at higher pH's in the 9.5–11.0 range. In addition, it is also known that peroxygen bleaches are most effective at higher temperatures, e.g., about 100° F. than at lower water temperatures. There is

now provided a detergent composition which has superior overall performance as a laundering and soaking composition. "Soaking composition" is a term used to describe a composition which is 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 bleach attack, 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 subtilius* 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 American 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 Apr. 5, 1967, and Ser. No. 748,806 filed July 30, 1968, both applications having been abandoned in favor of continuation-in-part application 844,656, filed July 24, 1969. These applications are incorporated herein by reference.

In describing the oxidizing bleach-containing detergent composition to which the present invention pertains, the description as given below is first of the enzyme component, then oxidizing bleach, the organic synthetic detergent, and lastly 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 and an oxidizing bleach is needed for bleaching purposes. A preferred embodiment is 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 deposition 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, p. 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, p. 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-City, Chiba-Prefecture.

(3) Applied Microbiology, March 1969, p. 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 *Streptomyes 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 organism 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 reaction, 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

	S. rectus var. *proteolyticus*
A. Morphological characteristics:	
Mycelium	Short branches form on a mono-axial basis.[1]
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 at 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 wrinkles.
Mycelium	Small quantity and white.
Soluble pigments	Initially yellow gold and later turn into red brown.
13. Starch-agar culture medium:	
Growth	Yellow brown, abundant and smooth.
Mycelium	Chalk white. Powder, and abundant.
Soluble pigments	Light yellow.
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.

TABLE I—Continued

	S. rectus var. *proteolyticus*
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	+ + +
Inositol	−
Control	−

[1] 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.

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—2%
Alkali extract of soybeans—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 III.

TABLE III

		Enzymatic activity [1]
	pH	Alkaline protease
Cultivation time:		
4	7.5	81
8	7.4	283
12	7.2	435
16	7.0	484

[1] 0.1 ml. of cultivation liquor.

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 precipitated 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 produce 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 V.

TABLE V

		Enzymatic activity [1]
	pH	Alkaline protease
Cultivation time:		
4	7.4	10
8	7.2	44
12	6.8	86
16	6.6	99

[1] O.D./ml. of the culture solution.

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, then 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. *proteolytic* ATCC 21067 can be achieved by practicing the procedures described in a study published in Applied Microbiology, March 1967, p. 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 suspensed 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 1μg. of tyrosine min., under the conditions specified.

Carboxypeptidase activity was assayed as follows: A 1 ml. reaction mixture (pH 8.0) containing 0.02 M carbobenzyoxy-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-cell 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. Akada, 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 expo-

nential 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 oxidizing bleach, detergents and builders described below in the proportions and 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 8,000,00 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.

OXIDIZING BLEACHING AGENTS

The oxidizing bleach embodiments of the present invention are so-called "peroxygen" bleaching agents, of which sodium and potassium perborate are the best known and most widely used. Other peroxygen compounds having bleaching power, however, also come within the scope of the present invention. For example, customary inorganic peroxy compounds such as other alkali metal perborates, percarbonates, and monopersulfates are useful. In addition, other examples are hydrogen peroxide and alkali metal peroxide such as sodium, potassium and lithium peroxide.

Sodium perborate as well as other peroxygen bleaches, while effective at higher temperature, e.g., boiling water temperature such as are commonly practiced in Europe and England, are somewhat less effective at the lower washing temperatures which are generally used in ordinary household laundering situations in the United States. Therefore, in order to boost the bleaching effectiveness of peroxygen bleaches, activators can be used. Such activators can be used in the compositions of the present invention without any detrimental effects. Example of especially effective bleach activators are triacetyl cyanurate (TACA), tetraacetyl methylene diamine (TAMD), sodium and potassium acetoxy benzene sulfonate (AOBS), phthalic anhydride, and benzoyl imidazole (BID).

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, especially 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 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 detergents is marketed under the trade name 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.

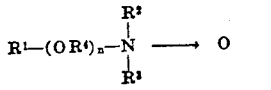
(2) Alkylphenol-polyethylene oxide condensates are condensation products of alkyl phenols having an alkyl group containing from about 6 to 12 carbon atoms in either a straight chain or branched chain configuration with ethylene oxide, the said ethylene oxide being present in amounts equal to 5 to 25 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 hydrophobic 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.

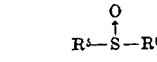
(6) Semi-polar nonionic detergents include long chain tertiary amine oxides corresponding to the following general formula



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 moonhydroxyalkyl 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. Pat. 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 ot two hydroxyl groups. Specific examples of these sulfoxides are: dodecyl methyl sulfoxide 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-dimethyl-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 Pat. 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, ampholytic 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.

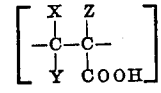
Useful alkaline organic builders are alkali metal, ammonium and substituted ammonium polyphosphonates, polyacetates and polycarboxylates. The polyphosphonates specifically include the sodium and potassium salts of ethylene diphosphonic acid, sodium and potassium salts or ethane-1-hydroxy-1,1-diphosphonic 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. Pats. 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:

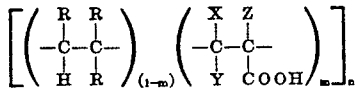


wherein X, Y, and Z are each selected from the group consisting of hydrogen, methyl, carboxyl, and carboxylmethyl, at least one of X, Y, and Z being selected from the group consisting of carboxyl and carboxymethyl, provided that X and Y can be carboxymethyl only when Z is selected from carboxyl and carboxymethyl, 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 monocarboxylic

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. Pat. 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 oxidizing bleach-containing alkaline detergent composition of this invention as described above and having the ingredients described herein of an organic synthetic detergent, an alkaline builder, an oxidizing bleach, and an enzyme elaborated from ATCC 21067 provides superior cleaning results at a pH in the range of 8 to 12, and preferably between 8.5 and 12. Below pH 8.0 overall cleaning performance of the composition begins to drop off. This is especially true in an embodiment in which a nitrilotriacetate builder is used. Above pH 12, little additional benefit is obtained and this is offset by considerations of the high alkalinity on mechanical parts of the washing apparatus.

The necessary pH can be readily achieved by proportioning the ingredients in the composition in such a way that the detergent, the builder, and the bleach provide the necessary pH at the recommended usage levels of the detergent composition. It is possible that within the above specified proportions of detergent, builder, and bleach, a detergent composition may not, per se, have a pH above 8.0 as required. In that event, the pH must be achieved or brought about either by reproportioning the detergent builder, and bleach, or, alternatively, adding to the composition or the washing solution, a pH adjuster or buffer, e.g., a suitable base such as sodium hydroxide, and the like, Ordinarily, the composition is readily formulated to meet the objective of having a pH in the range of 8.0 to 12. With the large number of alkaline builder materials described above, this presents no problem to those skilled in the art of formulating detergent compositions. To optimize the overall cleaning and stability results, it is preferred to practice the invention within the range of 8.5 to 12.

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 composition 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, bacteriostatic agents, dyes, optical brighteners, fluorescers, suds builders.

EXAMPLE

An alkaline detergent composition embodying the present invention contains:

	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
Water	9.0
Enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067	.1

This composition provides excellent overall cleaning and whiteness results when employed in a washing solution having a pH of about 9.7 and an enzyme concentration of 20 parts per million. The results are superior to a composition which contains only the enzyme or the sodium perborate bleach. Under these conditions an ordinary enzyme derived, for example, from a *Bacillus subtilis* organism is substantially degraded and overall cleaning is adversely effected.

In this example, the sodium nitrilotriacetate can be replaced by an equal amount of sodium tripolyphosphate or sodium pyrophosphate. An even more enzymatically effective alkaline composition is provided when the amount of enzyme is increased to 10% with adjustments made in the sodium tripolyphosphate and sodium sulfate levels.

This alkaline detergent composition can effectively be used as a soaking composition and also as a laundering detergent composition.

A preferred embodiment of the present invention is a soaking composition comprising from about 1% to 15% of an organic synthetic detergent, from about 55% to about 90% of an alkaline builder, from about 10% to about 40% of an oxidizing bleach agent and from about .05% to about 10% of a proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067. The detergent, builders, and bleach can be any of those mentioned and exemplified above.

The following composition is an example of a soaking composition which illustrates this preferred embodiment and which provides a pH of about 10.5. Excellent results are obtained at 10 parts per million.

EXAMPLE

	Percent
Tallow alkyl ethylene oxide condensate containing 30 ethylene oxide groups	4
Sodium tripolyphosphate	70
Sodium perborate	24
Proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067	2

This highly alkaline detergent composition can effectively be used as 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 perborate	10.0
Sodium sulfate	9.6
Water	6.0
Enzyme derifed from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067	.2

This composition is effective at a pH of 9.6 at a concentration of enzyme of 40 parts per million. It has excellnt stain-removing properties and enzyme 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 due to the bleaching effect of the perborate and the enzyme action.

EXAMPLE

This composition is an effective soil and stain-removing detergent at a pH 8.8 and a concentration of 5 p.p.m. of the enzyme component.

	Percent
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 perborate	5.0
Sodium silicate having an SiO_2:Na_2O ratio of 1.8:1	6.0
Coconut fatty acid ammonio amide	2.5
Sodium sulfate	9.0
Protease TP having 6% pure enzymes and a casein value of 400,000 units per gram	.2
Water	10.0

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, as well as soils which are susceptible to removal by an oxidizing bleaching agent.

The stain-removing properties of the enzyme-containing detergent compositions of the present invention were evaluated in the following manner.

Muslin swatches were stained by passing strips of muslin through a padding bath containing a staining solution, passing the muslin through a standard two-roll wringer and then drying the stained muslin strips. In order to effect deeper stains, the muslin strips were passed through the staining bath a second time followed by drying overnight at 120° F. The strip of muslin was then cut into swatches approximately 5 inches square in size. These stained swatches were then subjected to a laundering procedure which involved soaking the swatches in water containing a detergent composition for a predetermined amount of time and thereafter washing the swatches in a fresh supply of water. The washing step was done in an automatic miniature mechanical washer simulating an ordinary household laundering situation. The temperature of the soak and washing solutions were either at 75° F. or 140° F. as explained below, and had 7 grains hardness per U.S. gallon. The washing cycle was either 10 minutes or 15 minutes also as explained below. The pH of the solutions was 10. The laundering process was conducted by washing in a solution of the test composition a group of 12 swatches which was made up of two swatches each of the following five stains: gravy; egg; EMPA 112 which is a commercially available stain comprising cocoa and milk; EMPA 116 which is a commercially available stain comprising blood, milk and ink; and licorice. Two untreated terrycloth swatches were added to provide bulk to the wash load and made a total of 12 swatches.

The basic test composition was a conventional built anionic-containing detergent formulation and was employed in an amount of 4 grams per liter. The particular enzyme to be evaluated was added in the form of a fresh solution in water to the soak solution and the washing solution to provide the desired testing level of the enzyme component. Following the washing step, the swatches were dried and pressed and their whiteness and cleaning levels were measured using a Gardner Reflectometer. The specific instrument used was one manufactured by Gardner Laboratory, Inc., Bethesda, Maryland, Model AUX-HS-2 Reflectometer. This is an especially highly sensitive model in which high sensitivity is achieved by illuminating the washed sample with four beams instead of only two. The four beams not only increase illumination but also reduce the orientation effect of textured fabrics to a negligible amount. Mirrors are used to direct the beams onto the swatches. Illumination of the filter-photocell combinations by the perpendicularly reflected light is direct instead of indirect as in a less sensitive model. The greater the reflectance value the greater the cleaning whiteness. The soiled swatches were first graded prior to the soaking step and again at the completion of the washing and drying step. The difference in these two reflectance values was a measure of the stain-removing effectiveness of the enzyme and perborate-containing detergent composition. A measurement was also taken of stained swatches which were washed with the controlled detergent composition but without an enzyme and these values represented a control figure (referred to in the following tables as a "blank"). The controlled detergent composition employed to demonstrate the present invention was as follows:

Ingredients:	Percent by weight, percent
(1) Sodium dodecylbenzene sulfonate	21.6
(2) Sodium tripolyphosphate	21.6
(3) Sodium silicate having a SiO_2:Na_2O ratio of 2.0	4.9
(4) Sodium sulfate	28.3
(5) Sodium toluene sulfonate	2.2
(6) Sodium perborate tetrahydrate	10.0
(7) Water	9.24
(8) Carboxymethylcellulose	.9
(9) Miscellaneous (including brighteners)	1.26

In addition, the perborate-containing detergent composition also contained an amount of an enzyme component in an amount listed in the following tables. These tests were also repeated using different amounts of enzymes. The concentration of the enzyme component was either 30 p.p.m. as in Tables I and III or 70 p.p.m. as in Table II.

The values given in the following tables are reflectance values identified as "delta difference." As noted above, this difference is a measure of cleaning preformance and the higher the figure the more effective is that particular composition. The values given in the tables for the "blank" are those obtained by soaking and washing the

stained swatches only with the control detergent composition which did not contain an enzyme component. The procedure employed in Table I involved a presoaking time of 16 hours. (This is the equivalent of an overnight soak).

In each of the tests, comparisons were made between an enzyme of this invention, i.e., an enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067 and an enzyme derived from a *Bacillus substilis* organism, commercially available under the name ALCALASE. Alcalase is a commercially available alkaline proteolytic enzyme which is representative of those derived from the bacterial organism *Bacillus subtilis* and are termed subtilisins. This is a widely used commercial enzyme preparation. The specific subtilisin employed was the Carlsberg for which the amino acid sequence is described in Smith et al., "the complete amino acid sequence of two types are subtilisin, BPN' and Carlsberg," Journal of Biological Chemistry, volume 241, December 25, 1966, at page 5974. This particular subtilisin is characterized by a tyrosine to tryptophane ratio of about 13:1. This specific literature reference including its description of the amino acid sequence of the Carlsberg subtilisin (Alcalase) is hereby incorporated by reference. The Alcalase used is a fine grayish powder containing about 6% active enzyme. It has a particular size ranging from 1 mm. to 1.2 mm. and smaller, about 75% passing through a 100 mesh Tyler screen. The remainder of the powder being comprised primarily of sodium chloride, calcium sulfate, and various inert organic vehicle materials.

The analytical descriptions of the Alcalase enzyme and the Streptomyces-derived enzyme (Protease TP) were as follows:

Test or assay	Alcalase	Protease TP
Viable spore	1×10^8	3×10^4
Azocoll:		
pH 7.2, 30° C.	100	122
pH 9.3, 116° F.	100	82
Casein assay at -		
pH 7.0, 37° C.	227,000	369,000
pH 10.3, 37° C.	377,000	617,000
Anson hemoglobin, pH 7.5, 25° C.	1.44	2.55
Amylase assay, pH 6.0, 37° C. (Amylase units/gm.)	1,000	7,000
Skin flake hydrolysis, 25 p.p.m., .1% Tide, 7 gr./gal., 15 min. activity	100	165
Heat solution stability, half life in minutes, Azocoll substrate at pH 9.3, 130° F., 12 gr./gal.	26	37

A statistically significant visually observable difference in the grading values given in the following tables is .6 unit.

The improved stain removing results obtained with the perborate-containing detergent composition which contained .75% Protease TP can be seen from Table I. These improved results are indicative of the fact that the Protease TP enzyme is not inactivated by the sodium perborate bleach. By the same token, the lesser effectiveness of the Alcalase enzyme-containing composition is believed to be due to degradation of the enzyme by the perborate bleach. The effectiveness of Protease TP is especially noteworthy with the egg stain and the EMPA 116 stain.

The procedure employed in Table II was exactly that employed in Table I with the exception that the amount of each enzyme was increased to 7.75% thereby providing a concentration of enzyme in the washing solution of 70 parts per million. The superior stain-removing power of the Protease TP containing detergent composition is especially noteworthy with respect to the egg, EMPA 116 and EMPA 112 stains.

A similar procedure was employed to obtain the results presented in Table III except that the temperature of both the soaking solution and the laundering solution was 140° F. The soaking time was two hours and the laundering cycle was 15 minutes. Here again, the benefit of Protease TP stability in the presence of the sodium perborate is demonstrated. This is especially apparent in reviewing the reflectance values on the egg-stained and EMPA 116-stained swatches.

It is to be noted that the superior results demonstrated above with the detergent compositions prepared according to the present invention is appreciable because of the severe nature of the test. In Tables I and II, the enzyme-containing detergent composition was present throughout the 16-hour soaking step as well as the washing cycle. This means that the enzyme component was subjected to three of the most severe conditions which are known to inactivate enzymatic materials. These drastic conditions are high alkalinity, exposure to water, i.e., hydrolytic attack; and exposure to an oxidizing bleach, i.e., sodium perborate.

TABLE I

Enzyme sample (weight percent)	Concentration, p.p.m.	Gardner reflectance value (delta difference) *Egg	*EMPA 116	*EMPA 112	*Gravy
(1) Blank (no enzyme)		10.5	1.6	1.8	11.9
(2) .75% alcalase [1]	30	18.6	22.0	9.4	16.7
(3) .75% protease TP enzyme [2]	30	20.1	23.4	10.4	17.8

[1] As described previously.
[2] An enzyme preparation containing about 9% by weight active proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067 and balance inerts as described previously.
*Different stains.

TABLE II

Enzyme sample (weight percent)	Concentration, p.p.m.	Gardner reflectance value (delta difference) *Egg	*EMPA 116	*EMPA 112	*Gravy
(1) Blank (no enzyme)		10.5	1.6	1.8	11.9
(2) 1.75% alcalase	70	18.4	25.1	10.7	15.6
(3) 1.75% protease TP enzyme	70	20.4	36.1	13.8	15.7

*Different stains.

TABLE III

Enzyme sample (weight percent)	Concentration, p.p.m.	Gardner reflectance value (delta difference) *Egg	*EMPA 116	*EMPA 112	*Licorice
(1) Blank (no enzyme)		0	3.8	14.0	22.6
(2) .75% alcalase	30	6.2	8.2	15.4	27.4
(3) .75% protease TP enzyme	30	8.8	10.6	15.8	28.8

*Different stains.

The benefits obtained from a detergent composition containing both perborate and a Protease TP enzyme are seen from the results of the following demonstration simulating an ordinary household laundering situation.

In this demonstration, the same Protease TP sample was compared to the same Alcalase sample as described above in connection with the stain removal tests for which data is given in Tables I, II, and III. In addition, however, an amylase enzyme was added in order to discover any possible antagonistic results. Most commercially marketed detergent compositions contain a blend of different enzymes in order to provide a broader spectrum of enzymatic activity against different stains and soils. For this reason, Protease TP was evaluated with an amylase. The amplase enzyme used was a commercially available amylase enzyme preparation, Rapidase, having 500,000 amylase activity units. This is an enzyme preparation elaborated from a Bacillus subtilis organism and contains about 5% pure active enzyme. The balance is composed of inert organic and inorganic materials. Rapidase enzyme preparation is available from Rapidase, Seclin, France.

This demonstration involved a wash-wear test in which white shirts having detachable collars were worn by several male subjects. The collars were then randomly separated into several groups and the groups were then washed with a detergent composition being evaluated. The soil line on the worn collars served as a prime area for measuring the effectiveness of the composition. The fabrics after a soaking period, a washing and drying cycle were visually graded and these scores were then converted to a 1-10 grading scale.

The base composition used for this demonstration in an ordinary household cleaning application contained:

	Percent
Sodium dodecyl benzene sulfonate	17.40
Sodium tripolyphosphate	38.00
Sodium silicate	6.00
Sodium sulfate	17.18
Sodium toluene sulfonate	.60
Water	8.60
Carboxymethylcellulose	.45
Sodium perborate tetrahydrate	10.00
Miscellaneous	1.77

The soaking conditions were as follows: 16-hour soak; water 75° F., 7 gr./gal. hardness; 3/1 Ca/Mg hardness; 7.5 gal. water (about 7/1 water/cloth); .4% product concentration. The washing conditions were: 15 minutes agitation; 75° F. water, 7 gr./gal.; 3/1 Ca/Mg hardness; 15 gal. water (about 14/1 water/cloth), .4% product concentration; rinse water 75° F.

The detergent composition including the enzyme ingredients were preslurried in a small quantity of the soak or wash water for about 10 minutes before adding to the test solution.

Product A contained .7% Alcalase and .07% Rapidase both as described previously; Product B contained .7% Protease TP and .07% Rapidase also as described previously. The amount of Rapidase in both Products A and B was the same to avoid introducing any differences in the test compositions.

This elaborate test went through 12 cycles of wearing and soiling the fabrics followed by soaking, washing, drying and grading. The data so obtained was averaged and the results were that the base product with only perborate was graded as 3 (on a 1–10 scale), Product A was graded and, Product B, an embodiment of this invention, was graded as 8. The difference between 6 and 8 amounts to a statistically significant and visually noticeable difference.

It can be seen from the preceding description of the present invention that there is now provided a detergent composition that provides the advantages of an oxidizing bleaching agent and an enzyme without taking any extra processing and formulating steps to protect the enzyme. These compositions can be prepared readily as spray drying processes are preferred. In addition, they can be prepared by the technique of providing a carrier granule for the enzyme component according to the invention described in U.S. Pat. 3,451,935.

The preceding wash-wear demonstrates the usefulness of the present invention in an ordinary household washing situation. In addition to being effective against naturally soiled areas such as on shirt collars and cuffs, it was also simultaneously demonstrated that the composition of the present invention was equally effective in removing stains. To every other wash cycle in the preceding wash-wear test, two sets of stained swatches were added to each load for stain-removal determination. In other words, essentially the same stain-removal test described previously was made a part of this wash-wear test. The stained swatches were the same as those used in the previously described stain removal test. These included egg stains, EMPA 116 stains, EMPA 112 stains, gravy stains, and licorice stains. The grading procedure was the same as that described previously in connection with the stain-removal test. As in Tables I, II and III, a statistically significant, visually observable difference in the grading values is .6 units.

The results of this stain-removal test, run in conjunction with the wash-wear test, are presented in Table IV. Referring to Table IV, it will be seen that Product B, containing Protease TP and Rapidase, provided superior stain-removing results over the Alcalase/Rapidase composition, Product A. Product B, representing a preferred embodiment of the present invention, was especially effective on the egg stain and the EMPA 116 stain.

TABLE IV

Enzyme sample	Concentration, p.p.m.	Garner Reflectance Value (delta difference) *Egg	*EMPA 116	*Gravy	*EMPA 112	*Licorice
(1). No E-base		0	0	0	0	
(2). Product A [1], alcalase/rapidase	28/2.8	20.4	26.6	9.6	12.2	10.4
(3) Proudct B [1], protease TP/rapidase	28/2.8	21.3	35.7	9.4	12.2	10.7

[1] As described previously in conjunction with the stain-removal test for which data is given in Tables I, II, and III.
*Different stains.

Besides those enzymes mentioned above, others can also be added to provide detergent compositions having even greater enzymatic activity including other proteases, amylases and lipases. Thus, for instance, it is possible to optionally add pepsin, tryptin, chymotrysin, 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), Papain 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 (Rhom and Haas).

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

It has also been discovered that the proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067 is unexpenctedly 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 an enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067 provides unique and unexpected advantages when employed in a highly alkaline detergent composition. Such an invention is described and claimed in a copending patent application filed by Siebert et al. entitled "Highly Alkaline Detergent Composition Containing An Enzyme Derived from Thermophilic *Streptomyces rectus* var. *proteolyticus*." This latter invention is embodied in 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 21067, said composition having a pH in excess of 9.5 and in the pH range of 9.5 to 11. The aforementioned copending patent application is incorporated herein by reference. This invention is embodied in the following illustrative example:

	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.

As noted above, the present invention is based upon the unique properties of the proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067. The thermophilic Streptomyces organism has been described above in essential detail. However, a more complete description of this organism together with a description of a process for producing an enzyme by this organism is found in separate copending patent applications Ser. No. 628,725, filed Apr. 5, 1967, and Ser. No. 748,806, filed July 30, 1968. These applications, together with those previously mentioned, are incorporated herein by reference.

It is pointed out that while the preceding description has been concerned primarily with the problem caused by the degradation of an enzyme by an oxidizing bleach, it is equally necessary that the enzyme not be deterimental to the oxidizing bleach and impair its usefulness. In this invention, these highly sensitive components are compatible in all respects.

While thermostability of the Protease TP enzyme is excellent and while higher temperature seem to improve enzyme activity until a lysis stage is reached; a surprising aspect of this invention is that the Protease TP enzyme is also effective at lower temperatures, e.g., less than 100° F., and as low as 50° F. This permits formulation of a product containing enzyme and sodium perborate which is useful for soaking at room temperatures and also hand washing of soiled garments. For example, such a composition can contain:

	Percent
Sodium dodecyl benzene sulfonate	27
Sodium tripolyphosphate	27
Sodium perborate	10
Protease TP	.6
Sodium silicate	6
Sodium sulfate	20
Water	9.4

This composition provides a pH of 9 in solution and is an effective bleach-containing and enzyme-containing detergent composition.

Another example of an effective enzyme and bleach-containing composition was:

	Percent
Sodium dodecyl (straight-chain) benzene sulfonate	10
Tallow (ethylene oxide)$_3$ condensate	3
Sodium tripolyphosphate	32
Sodium perborate	30
Protease TP	.5
Sodium silicate	6
Water	8.5
Sodium sulfate	10

This composition is an excellent soak and washing composition which provides a pH of 9.8 to 10.1. For soaking a product concentration of .4–.6% is desired; for main wash a product concentration of .6–.9% is desired.

The percentages and proportions given herein are by weight unless otherwise specified.

The foregoing description of the present invention has been presented describing certain operable and preferred embodiments. It is not intended that the invention should be so limited since variations and modifications thereof will be obvious to those skilled in the art, all of which are within the spirit and scope of this invention.

What is claimed is:

1. A detergent composition consisting essentially of an organic synthetic detergent selected from the group consisting of anionic, nonionic, zwitterionic, and ampholytic synthetic detergents and mixtures thereof and an organic or inorganic alkaline builder salt in a weight ratio of from about 10.1 to about 1:30 from about 2.5% to about 60% of an inorganic peroxygen bleaching agent and from about .0025% to about 10% of a proteolytic enzyme derived from thermophilic *Streptomyces rectus* var. *proteolyticus* ATCC 21067, said composition being effective in a pH range of 8–12.

2. A detergent composition according to claim 1 in which said bleaching agent is present at from about 10% to about 50%.

3. A detergent composition according to claim 2 in which said bleaching agent is sodium or potassium perborate.

4. A detergent composition according to claim 1 in which said proteolytic enzyme is present in an amount of from about .0025% to about 3%.

5. A detergent composition according to claim 1 in which the ratio by weight of said detergent to said builder is in the raneg of about 5:1 to about 1:20.

6. A process for washing stained fabrics which comprises immersing said stained fabrics in an aqueous solution containing the detergent composition of claim 1 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.

7. A process according to claim 6 in which the concentration of said proteolytic enzyme is in the range of from about 1 to about 60 p.p.m.

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