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(54) Lipase and cutinase surfactant systems and method useful in laundering.

(57) The activation of glycerol ester hydrolases, toward triglyceride oils, is correlated to the molar ratio of total triglyceride to surfactant concentrations in the test solution. This ratio dependency, when considered in laundry compositions, allows for predictable and improved utilization of these enzymes in the hydrolysis of triglyceride stains. A particularly preferred enzyme for this use is isolatable from *Pseudomonas putida* ATCC 53552. This enzyme, or hydrolase, has sufficient hydrolysis activity in a laundering solution to hydrolyze at least about 5 wt. % of total triglyceride stains in a laundering solution within about 15 minutes at about 25 °C.

LIPASE AND CUTINASE SURFACTANT SYSTEMS AND METHOD USEFUL IN LAUNDERING

The present invention relates to a method to optimize enzymatic detergent systems useful in cleaning, a predictive method to identify preferred laundry and cleaning applications, and compositions based thereon.

Lipases are enzymes naturally produced by a wide variety of living organisms from microbes to higher eukaryotes. Fatty acids undergoing oxidation in tissues of higher animals must be in free form (that is, non-esterified) before they can undergo activation and oxidation. Thus, intracellular lipases function to hydrolyze the triacylglycerols to yield free fatty acids and glycerol.

Bacterial lipases are classically defined as glycerolesterhydrolases (EC 3.1.1.3) since they are polypeptides capable of cleaving ester bonds. They have a high affinity for interfaces, a characteristic which separates them from other enzymes such as proteases and esterases. An interface onto which lipases readily absorb is that of oil/water.

Cutinases are esterases that catalyze the hydrolysis of cutin. For example, cutinase allows fungi to penetrate through the cutin barrier into the host plant during the initial stages of a fungal infection. The primary structures of several cutinases have been compared and shown to be strongly conserved. Ettinger, Biochemistry, 26, pp. 7883-7892 (1987). Sebastian et al., Arch. Biochem. Biophys., 263 (1), pp. 77-85 (1988) have recently found production of cutinase to be induced by cutin in a fluorescent *P. putida* strain. This cutinase catalyzed hydrolysis of p-nitrophenyl esters of C_4-C_{16} fatty acids.

Lipases have long been considered as potential components in detergent compositions. An early preparation of lipase in the form of pancreatin was taught for addition to detergent formulations by Rohm, Chem. Abs., Int., P2048 (1916). More recently, lipases obtained from certain *Pseudomonas* or *Chromobacter* microorganisms have been disclosed as useful in detergent compositions: Thom et al., U.S. Patent No. 4,707,291, issued November 17, 1987 and Wiersema et al., European Patent Application 253,487, published January 20, 1988.

Lipases have long been known generally to be inhibited by anionic detergents and by nonionic detergents. Lipase activity has been reported as enhanced by emulsifiers such by Wills, Bioch., 60, pp. 529-534 (1955) and Andree, et al., J. App. Biochem., 2, pp. 218-229 (1980). Not withstanding such teachings, attempts to use lipases in laundry solutions which contain anionic or nonionic surfactants have been largely unsuccessful, and effective use of lipases for cleaning oily stains have been limited to presoak applications.

U.S. Patent 3,950,277, inventors Stewart et al., issued April 13, 1976 describes pre-soak compositions employing a lipase enzyme and a lipase activator selected from the group consisting of naphthalene sulfonates, certain polyoxyalkylene derivatives of ethylene diamine and certain acylamino acid salts.

Lipases have been found useful in aqueous solution, without added surfactants, for prewash or presoak applications over extended periods of time, followed by a conventional washing with fully formulated detergents. Under these conditions, lipases have been effective in removing natural oil (triglyceride) stains. But despite the many attempts to use lipase in detergent formulations for laundering solutions, the demonstrated washing benefit has been disappointing.

Attempts have recently been made to find particular lipases that are less affected by detergents in wash solutions. European Patent Application 258,068, published March 2, 1988 reports a lipase from the genus *Thermomyces* said to be compatible with anionic surfactants and effective as a detergent additive. Japanese Patent Application 63039579, published February 20, 1988 states that a novel lipase, obtained from a *Pseudomonas*, is only slightly inhibited by anionic surfactant and is activated by nonionic surfactant.

In summary, there have been no clear teachings on the compatibility or incompatibility of lipases in laundry and cleaning formulations, although it has been generally recognized that specific surfactants (when present in useful amounts in detergent formulations) will inhibit lipase activity for some lipases. As a consequence, laundering solutions including lipases have tended to be those that require extended soaking.

Summary of the Invention

It is an object of the present invention to provide enzyme compositions, useful in surfactant laundering solutions, having an enzyme capable of hydrolyzing triglyceride on fabric and an additive that will prevent inhibition of enzymatic hydrolysis by the surfactant in the solution, and thus allowing the onset of hydrolysis of the triglyceride fabric soil or stain.

It is another object of the present invention to provide a method for defining lipase compatibility, or incompatibility, in laundering solutions so that surfactant systems may be formulated that include lipases and/or cutinases for use in laundering solutions, without requiring extended soaking or high temperatures for triglyceride hydrolysis.

In one aspect of the present invention, an enzyme composition for laundry applications comprises an enzyme capable of hydrolyzing natural oil stains on fabric when in a laundry solution and hydrolysis activating means for avoiding inhibition of such hydrolysis by surfactants in the laundry solution. Thus, the onset of hydrolysis for such an enzyme is dependent upon exceeding a critical ratio. It has been discovered that such enzymes will "turn on" and hydrolyze the oil stain only if the molar ratio of oil to surfactant in the laundry solution, at the oil stain interface, exceeds a certain value, referred to herein as the "critical ratio". The value of the critical ratio for each enzyme depends upon the identity of surfactant used.

The hydrolysis activating means changes the ratio of oil to surfactant in a laundry solution in which the composition is employed to exceed the critical ratio, so that the enzyme will "turn on" and hydrolyze the oil stain.

Alternatively, another embodiment of the invention provides hydrolysis activating means for changing the critical ratio of the surfactants in the laundry solution.

The enzymes of the inventive composition (in combination with hydrolysis activating means for changing either the ratio of oil to surfactant or the critical ratio of surfactant) has sufficient hydrolytic activity in a surfactant laundering solution to hydrolyze at least about 5 wt.% of the total oil stain, such as triglyceride, in a laundering solution within about 14 to 15 minutes at about 25° C.

A particularly preferred enzyme for use in the present invention is isolatable from *Pseudomonas putida* (hereinafter *"P. putida"*) ATCC 53552.

In another aspect of the present invention, a method for selecting a surfactant system with which inhibition of a lipase or cutinase is reduced comprises providing an aqueous solution having a lipase or a cutinase, a surfactant and an oil, calculating a molar ratio of oil to surfactant in the aqueous solution and comparing the calculated ratio to a predetermined ratio to determine whether the lipase or cutinase will have hydrolysis activity in the presence of the surfactant.

Other aspects and advantages of the invention will become apparent to those skilled in the art upon reading the following description.

Detailed Description of the Preferred Embodiments

Fabrics cleaned in laundering solutions include clothing soiled by body oils (sebum) and linens soiled by food and cooking oils. Mono-, di- and triglycerides are present in sebum soils and cooking oils and potentially can be hydrolyzed by lipases.

Analysis of the amino acid sequence for a recently discovered enzyme described as having lipase activity and isolatable from *P. putida* ATCC 53552 suggests there are substantial homologies between the nucleotide sequence for this enzyme and the nucleotide sequence of the cutinase gene recently determined for *C. capsici.* (Compare European Patent Application 268,456, inventors Wiersema et al., published May 25, 1988 with Ettinger et al., Biochemistry, 26, pp. 7883-7892 (1987)). Because of the relationship between cutinases and lipases, enzymes of interest for the present invention include both cutinases and lipases which are capable of hydrolyzing triglyceride on fabric in aqueous solution and will sometimes hereinafter be described as glycerol ester hydrolases. Such enzymes useful in the present invention are typically obtained from certain *Pseudomonas*, *Chromobacter*, *Fusarium* or *Aspergillus* strains. For example, among the enzymes with this invention has been listed are those expressed by genes present in (or obtainable from) *P. putida* ATCC 53552, from *P. sp.* (as Amano 68S), from *P. fluorescens* (as Amano P), and from *Aspergillus oryzae* (as Lipolase). Toyo Jozo Co. of Japan, U.S. Biochemical Co. of the U.S.A. and Diosynth Co. of The Netherlands sell lipases from *Chromobacter viscosum.* European Patent Application No. 0,214,761, published March 3, 1987, applicant Novo Industri, describes a lipase from *Fusarium oxysporum.* Yet other strains are known or described as producing lipases. For example, PCT/WL86/00023, published February 12, 1987, applicant Gist-Brocades N.V., describes strains including certain *Acinetobacter.* It should be understood that the genes expressing such enzymes can be cloned into another organism such as *E. coli*, for higher levels of expression.

CRITICAL RATIO

Using a critical ratio as described herein, we have discovered that when in a surfactant laundering solution, the hydrolysis of oils, having ester bonds, by specific glycerol ester hydrolases in the presence of surfactant is dependent upon the ratio of oil to surfactant in the solution of interest. The ratio of oil to surfactant in the solution of interest will sometimes hereinafter be called the "system ratio". For convenience, both this system ratio and the value of the ratio required for hydrolysis to begin (called the "critical ratio") will be calculated and expressed on the basis of the molar relationship of oil to surfactant in the bulk aqueous solution. The system ratio defines the relationship of substrate (oil) with respect to specific surfactant on an absolute mole/mole basis. At the critical ratio and above the molar relationship of substrate with respect to a specific surfactant is such that the enzyme is activated, or "turned on", such that hydrolysis begins. That is, concentration of either component in an aqueous medium, such as wash water solution, is not critical because the molar relationship between the two components remains constant in spite of dilution in the aqueous medium.

Without being restricted to a single theory, it may be that the enzyme has difficulty binding the substrate unless the substrate is changed in some way facilitated by surfactant. That is, there appears to be a necessary complex between substrate and surfactant formed in order for the substrate to be hydrolyzable by enzyme.

In the examples which follow, different lipases and cutinases are shown to have related, but different, critical ratios, which applicants have determined empirically through their above-described model. One key aspect of the critical ratio model is a focus upon the amount of substrate present relative to the amount of surfactant, rather than on the amount of enzyme or surfactant. By contrast, the prior art has assumed that either using large amounts of enzymes, or surfactants, or repeatedly testing with either or both, will result in enhanced cleaning performance. But following teachings or assumptions of the such prior art leads one to using wastefully large amounts of enzyme, surfactant, or both.

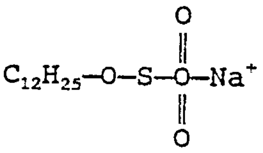
The molecular weights of various surfactants (and typical structures) and oils discussed hereinafter are set out below.

Compound	Nominal Molecular Weight*
SDS (sodium dodecylsulfate) surfactant	288
C_{12}LAS surfactant	362
Neodol 25-9 surfactant	596
Neodol 25-3S surfactant	444
Surfonic JL80X surfactant	625
Triton X-100 surfactant	624
C_{16}DAPS surfactant	392
Trioctanoin substrate	470
Triolein substrate	884

* Used for calculations of molarity, and calculated as if for pure surfactant.

Typical structures of common surfactants are:

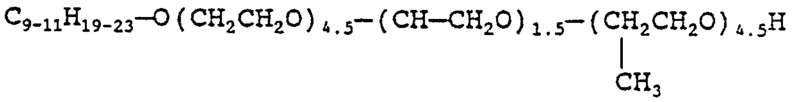
SDS



$$C_{12}H_{25}-O-S-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{O}}-Na^{+}$$

C_{12}LAS $C_{12}H_{25}\text{-}\emptyset\text{-}SO_3^{-}Na^{+}$
Neodol 25-9 (C_{12}-C_{15}) (H_{25}-H_{31})-$(OCH_2CH_2)_9$-H
Neodol 23-6.5 (C_{12}-C_{13}) (H_{25}-H_{27})-$(OCH_2CH_2)_{6.5}$-H

Surfonic JL80X

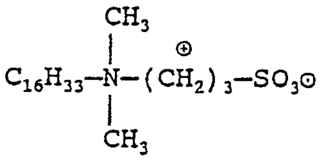


$$C_{9-11}H_{19-23}-O(CH_2CH_2O)_{4.5}-(CH-CH_2O)_{1.5}-(\underset{\underset{CH_3}{|}}{CH_2}CH_2O)_{4.5}H$$

Triton X-100

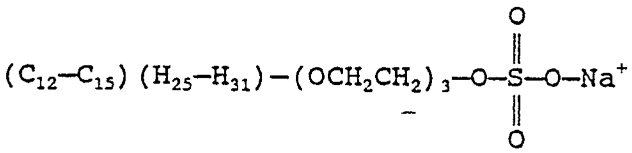
$C_8H_{17}-\text{Ø}-(OCH_2CH_2)_{10}-H$

C_{16}DAPS



$$C_{16}H_{33}-\overset{\overset{CH_3}{|}}{\underset{\underset{CH_3}{|}}{N}}{}^{\oplus}-(CH_2)_3-SO_3^{\ominus}$$

Neodol 25-3S



$$(C_{12}-C_{15})(H_{25}-H_{31})-(OCH_2CH_2)_3-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}-O-Na^+$$

Initiation of substrate hydrolysis by the glycerol ester hydrolases depends strongly on the system ratio and not on the concentration of either substrate (triglyceride) or surfactant.

An example of the effect of the system ratio on hydrolysis of the substrate by a glycerol ester hydrolase is illustrated by Table IA where enzymatic activity was monitored for a number of different trioctanoin concentrations at two different surfactant concentrations. The surfactant used for the Table IA data was a zwitterionic salt sometimes abbreviated C_{16}DAPS ("Zwittergent 3-16" available from Calbiochem).

TABLE IA

Oil[1] Conc. (mM)	Surfactant[2] Conc. (mM)	System Ratio	Enzyme Activity[3]
0.5	0.5	1	0
10	10	1	0
2.5	0.5	5	0
50	10	5	0
5	0.5	10	0
10	0.5	*20	334
200	10	*20	398
15	0.5	30	405
300	10	30	370
20	0.5	40	370
400	10	40	417

[1] Trioctanoin

[2] Zwittergent 3-16

[3] initial rates of hydrolysis measured as µmole H^+ released • min^{-1} • mgE^{-1} of enzyme from *P. putida* ATCC 53552 (2 ppm)

* Indicates the critical ratio exceeded

As may be seen from the data of Table IA, there is either no enzyme activity (that is, the enzyme is "turned off") or observable hydrolyase activity, depending upon the system ratio, and independent of the surfactant concentration. This data shows that neither the absolute concentration of the triglyceride nor the absolute concentration of the surfactant determines whether or not the enzyme is active. Instead, it is the ratio of the oil to surfactant that best describes the kinetic profile of enzyme activity. For the enzyme tested in Table IA, the value of the critical ratio (i.e. system ratio at which enzymatic activity begins) with respect to this particular zwitterionic surfactant is between 10 and 20. At and below the system ratio value of 10, the enzyme is not active. At and above the system ratio value of 20, the enzyme is active.

Another example of this phenomenon, using another surfactant with which the *P. putida* ATCC 53552 enzyme displays a different critical ratio, sodium oleate, is presented in Table IB.

TABLE 1B

Na Oleate (mM)	System Ratio	Enzyme Activity
0.3	1	0
0.3	5	0
0.3	10	0
0.3	20	60
0.3	30	90

The substrate used for the data of Table IB was triolein. The concentrations (not shown) were varied to produce the system ratios indicated. The sodium oleate surfactant used in the experiment summarized by Table IB is interesting because oleic acid is a product of reaction hydrolysis.

The experiments determining enzyme activity, such as those set out in Tables IA and IB, were carried out as follows:

(i) Sample Preparations:

The desired amount of triglyceride was weighed into an appropriate size beaker, on a Mettler balance (model number AE163). The corresponding amount of surfactant was added to the triglyceride, from a previously prepared aqueous surfactant stock solution, and the triglyceride and surfactant mixed manually. The sample was then adjusted to the desired weight using doubly distilled H_2O. Emulsification of the sample was carried out, prior to assaying enzyme activity, with a probe sonicator (Braun-Sonic model 2000), on ice, for approx. 2 minutes.

(ii) Enzyme Activity Measurement:

This was achieved by monitoring the rate of acid liberation, from the enzymatic hydrolysis of the triglycerides in the emulsion. The assay was initiated by adding approx. 2ppm lipase to 10 ml of the prepared emulsion. The acid liberated was monitored by autotitration, on a Radiometer pH-stat (model number ABU80) to an endpoint of a pH of 10. Initial rates were recorded for the first 5 minutes of the reaction, and the reaction rates reported as μmole H^+ titrated . min $^{-1}$mgE^{-1}. Occasionally enzyme activity is reported as % total oil hydrolyzed in 14 min. In these examples, the reaction was allowed to run for 14 min. and the amount of acid titrated recorded. The % total oil (triglyceride) hydrolyzed was then calculated by dividing the recorded value with the theoretically calculated value assuming three equivalents of oleic acid was produced for each triglyceride equivalent. All assays were run at ambient temperatures.

The dependency of the onset of hydrolysis upon a critical ratio of oil to surfactant in aqueous solution is not specific to the particular glycerol ester hydrolase used for the data of Table IA and Table IB; rather, the principle has been discovered to be general for other glycerol ester hydrolases. This is shown by Tables II-V, which show the critical ratio for a variety of different nonionic and anionic surfactants and several different enzymes (where the substrate was trioctanoin). The various enzymes examined as shown by Tables II-V were also examined at higher surfactant concentrations and the dependency upon the system ratios was confirmed.

TABLE II

(Enzyme isolatable from *P. putida* ATCC 53552)

Surfactant Type & Conc.	Critical Ratio	Enzyme Activity*
Anionic[1], 2 mM	0.5 - 5.0	325
Anionic[2], 1 mM	5 - 10	250
Anionic[3], 2 mM	0.5 - 1	300
Nonionic[4], 0.5 mM	10 - 20	450
Nonionic[5], 0.5 mM	5 - 10	500
Nonionic[6], 2 mM	10 - 15	450

[1] SDS (sodium dodecylsulfate)
[2] C_{12}LAS (available from Pfaultz and Bauer Inc.)
[3] Neodol 25-3S (available from Shell)
[4] Neodol 25-9 (available from Shell)
[5] Surfonic JL-80X (available from Texaco)
[6] Triton X-100 (available from Rohm and Haas)
*As described by footnote 3 in Table IA.

TABLE III

(Enzyme Amano P., available from Amano Co., isolatable from *Pseudomonas fluroescens*)

Surfactant Type & Conc.	Critical Ratio	Enzyme Activity*
Anionic[1], 2 mM	1 - 5	100
Anionic[2], 1 mM	5 - 20	70
Anionic[3], 2 mM	10 - 20	200
Nonionic[4], 0.5 mM	1 - 5	400
Nonionic[5], 0.5 mM	0.5 - 1	550

[1] SDS
[2] C_{12}LAS
[3] Neodol 25-3S
[4] Neodol 25-9
[5] Surfonic JL-80X
*As described by footnote 3 in Table IA.

TABLE IV

(Enzyme Amano 68S, available from Amano Co., isolatable from *P. sp.*)		
Surfactant Type & Conc.	Critical Ratio	Enzyme Activity*
Anionic[1], 1 mM	0.1 - 0.5	400
Anionic[2], 0.2 mM	5 - 10	175
Anionic[3], 0.5 mM	1 - 5	200
Nonionic[4], 0.5 mM	≦ 0.1	750
Nonionic[5], 0.5 mM	0.5 - 1	700

[1] SDS

[2] C_{12}LAS

[3] Neodol 25-3S

[4] Neodol 25-9

[5] Surfonic JL-80X

*As described by footnote 3 in Table IA.

TABLE V

(Enzyme Lipolase, available from Novo Industri, isolatable from *A. oryzae*)	
Surfactant Type & Conc.	Critical Ratio
Anionic[2], 0.5 mM	0.5 - 1
Anionic[3], 0.5 mM	20 - 30
Nonionic[4], 0.5 mM	20 - 30
Nonionic[5], 0.5 mM	10 - 20

[2] C_{12}LAS

[3] Neodol 25-3S

[4] Neodol 25-9

[5] Surfonic JL-80X

As can be seen by Tables I-V, the critical ratios for particular enzymes are dependent upon surfactant identity.

The following Tables VI-IX show that hydrolysis is also dependent upon substrate type. The data of Tables VI-IX was collected using triolein as the oil (rather than trioctanoin as in Tables I-V).

TABLE VI

(Enzyme from *P. putida* ATCC 53552)		
Surfactant Type & Conc.	Critical Ratio	Enzyme Activity*
Anionic[1], 1 mM	5 - 10	60
Anionic[2], 0.5 mM	5 - 10	50
Anionic[3], 1 mM	1 - 5	125
Nonionic[4], 0.5 mM	10 - 20	60
Nonionic[5], 0.5 mM	0.5 - 1.0	150

[1] SDS
[2] C_{12}LAS
[3] Neodol 25-3S
[4] Neodol 25-9
[5] Surfonic JL-80X
*As described by footnote 3 in Table IA.

TABLE VII

(Enzyme Amano P)		
Surfactant Type & Conc.	Critical Ratio	Enzyme Activity*
Anionic[1], 1 mM	5 - 10	160
Anionic[2], 0.5 mM	10 - 20	13
Anionic[3], 0.5 mM	5 - 10	20
Nonionic[4], 0.5 mM	5 - 10	20
Nonionic[5], 0.5 mM	5 - 10	40

[1] SDS
[2] C_{12}LAS
[3] Neodol 25-3S
[4] Neodol 25-9
[5] Surfonic JL-80X
*As described by footnote 3 in Table IA.

TABLE VIII

(Enzyme Amano 68S)		
Surfactant Type & Conc.	Critical Ratio	Enzyme Activity*
Anionic^1, 1 mM	1 - 5	200
Anionic^2, 0.5 mM	10 - 20	30
Anionic^3, 0.5 mM	5 - 10	25
Nonionic^4, 0.5 mM	1 - 5	30
Nonionic^5, 2.5 mM	1 - 5	40

[1] SDS
[2] C_{12}LAS
[3] Neodol 25-3S
[4] Neodol 25-9
[5] Surfonic JL-80X
*As described by footnote 3 in Table IA.

TABLE IX

(Enzyme Lipolase)	
Surfactant Type & Conc.	Critical Ratio
Anionic^2, 0.5 mM	30-40

[2] C_{12}LAS

The above data may be summarized by the "Table Summary" below where "++" means a critical ratio of 0.01 - 0.1, "+" means a critical ratio of 0.1-1.0, "0" means a critical ratio of 1.0-10, and "-" means a critical ratio of 10-100.

TABLE SUMMARY				
	Enzyme			
(Trioctanoin as substrate) Surfactant	From ATCC 53552	Amano P	Amano 68S	Lipolase
Anionic[1], 2 mM	0	0	+	ND
Anionic[2], 1 mM	0	0	0	+
Anionic[3], 2 mM	+	-	0	-
Nonionic[4], 0.5 mM	-	0	+ +	-
Nonionic[5], 0.5 mM	0	+	+	-
Nonionic[6], 2 mM	-	ND	ND	ND

[1] SDS (sodium dodecylsulfate)

[2] C_{12}LAS (available from Pfaultz and Bauer Inc.)

[3] Neodol 25-3S (available from Shell)

[4] Neodol 25-9 (available from Shell)

[5] Surfonic JL-80X (available from Texaco)

[6] Triton X-100 (available from Rohm and Haas)

ND = Not Determined

(Triolein as substrate) Surfactant	From ATCC 53552	Amano P	Amano 68S	Lipolase
Anionic[1], 2 mM	0	0	0	ND
Anionic[2], 1 mM	0	-	-	-
Anionic[3], 2 mM	0	0	0	ND
Nonionic[4], 0.5 mM	-	0	0	ND
Nonionic[5], 0.5 mM	+	0	0	ND

[1] SDS (sodium dodecylsulfate)

[2] C_{12}LAS (available from P. Faultz and Bauer Inc.)

[3] Neodol 25-3S (available from Shell)

[4] Neodol 25-9 (available from Shell)

[5] Surfonic JL-80X (available from Texaco)

USE RATIO

The four enzymes tested (Tables I-IX) all demonstrated a critical ratio for each of the surfactants tested. These surfactants constitute some of the most commonly used surfactants in commercially available detergent compositions. Such detergent compositions are typically recommended for United States laundering use in amounts that, when dissolved in laundry solution, provide a surfactant concentration between about 0.2 mM and about 1.5 mM (assuming a 2-3 kg average load in a 72 liter wash solution).

The average amount of oily soil on fabrics in household laundries is an estimated 300 mg oil/100 g of fabric (Andree et al., J. App. Biochem, 2, pp. 218-229 (1980). This indicates that, based on the ratio dependency demonstrated above, inclusion of lipases in most commercially available detergents would provide little or no washing benefit because the use ratio (of actual oil concentration to actual moles of surfactant in the laundering solution) is below the critical ratio at which enzyme activity is initiated. The situation is similar for Europe and Japan because, although the fabric load, wash solution and recommended detergent usage differ from the United States, the use ratios are typically less than about 0.6 for Japan and less than about 0.4 for Europe.

That is, based on the recommended detergent use and considering a wide variety of detergent compositions and surfactant molecular weights, the system ratios for most common detergents are typically less than 1, more usually on the order of about 0.2-0.6. (In calculating the use ratios, the bulk concentrations in solution have been assumed and any possible interfacial effects ignored.) But as can be seen from the data of Tables II-IX, the critical ratio for the common surfactants studied (with trioctanoin as oil) are generally greater than about 1. The performance at use ratios below the critical ratio has made attempts to include lipases in laundering solutions generally ineffective.

Typical detergent compositions for laundering include various additives, such as builder salts. It has been discovered that at use levels the additives commonly utilized in detergents have no substantial effect on the critical ratio (data not shown).

Use of combinations of surfactants having different critical ratios can generate critical ratios that are different from the individual surfactants in the combination. By practicing the invention, one can admix surfactants to achieve a critical ratio of the combined surfactants that is at or below the lower of the individual critical ratios. This will be further explained hereinafter.

One conventional detergent composition is a mixture of Neodol 25-3S and C_{12}LAS (with a molar ratio of 1:0.4). This conventional detergent composition exemplifies the difficulties encountered in prior attempts to include lipases in laundering solutions. By examination of the appropriate data for the component surfactants of the detergent composition in Table VI, one could conclude that the critical ratios are much higher than the use ratio. This conclusion proves true when a swatch study and a washing machine study were conducted, as illustrated by the data of Table X, where a solution included either the conventional detergent or the conventional detergent plus ATCC 53552 enzyme.

TABLE X

Treatment	Use Ratio[1,2]	Stain Removal[2,3]
14 min. washes (Neodol 25-3S and C_{12}LAS, 1:0.4 molar ratio), 5 min. rinse	0.05	49.61
14 min. washes (Neodol 25-3S and C_{12}LAS, 1:0.4 molar ratio), and ATCC 53552 enzyme, 5 min. rinse	0.05	51.07

[1] Detergent comparisons at 1.29 g/l; enzyme, when present, at 2µg/ml.
[2] Stain: Cotton swatches with sebum soil (Synthetic)
[3] % SR(E), as described below.

As illustrated by the data of Table X, the stain removal value of the detergent composition with enzyme was not statistically different from the stain removal value with the detergent composition without enzyme. Thus, the enzyme was substantially not active. Calculation of the use ratio shows the use ratio was below the determined critical ratio of 10-20, and thus the enzyme was inactive.

As earlier noted, soil removal was measured on a stain removal scale designated "%SR(E)". This is a scale expressing the ratio of the change of appearance of a soiled, treated test sample to its maximum possible change of appearance.

Values of %SR(E) are calculated as follows:

$$\%SR(E) = \frac{\Delta E_S - \Delta E_{OW}}{\Delta E_S} \times 100$$

where E_S and E_{OW} are distances in the CIE L*a*b* color space [see, Hunter, *The Measurement of Appearance* (New York: John Wiley & Sons, 1975) pp. 302-303.] and are given by

$E_S = \sqrt{(L^*_o - L^*_s)^2 + (a^*_o - a^*_s)^2 + (b^*_o - b^*_s)^2}$

$E_{OW} = \sqrt{(L^*0 - L^*W)^2 + (a^*0 - a^*W)^2 + (b^*0 - b^*W)^2}$

in which the subscripts o,s, and w refer to the original unstained and untreated test sample, the stained and the untreated test sample, and the stained and treated test sample, respectively.

The statistical test denoted as the "LSD" refers to the smallest difference between within-group means that would be declared statistically significant at the 95% confidence level using the two-sample test *t*-test with the variance estimated from all groups in the analysis of variance.

As a brief summary then, enzymes capable of hydrolyzing natural oil stains on fabric when in a laundry solution have been shown to have a dependency for the onset of hydrolysis upon a critical value of the molar use ratio of oil to surfactant in the laundry solution. This critical ratio is dependent upon the type of surfactant in the laundering solution (and also upon the type of oil in the laundering solution). But because the use ratios for most common detergents are typically less than 1 and the critical ratio for the common surfactants studied are generally greater than about 1, lipases generally are inactive.

However, we have discovered ways of "turning on" hydrolysis by the enzyme through hydrolysis activating means for changing the ratio of oil to surfactant or for changing the critical ratio of the surfactants. Examples of ways to modify the critical ratio will now be more fully described. In addition, compositions of the invention may be beneficially formulated with more than one lipase or cutinase.

INCREASING THE SYSTEM RATIO WITH ADDITION OF OILS OR OTHER ORGANIC COMPOUNDS

Hydrolases can be "turned on" in the presence of a surfactant by the addition of an oil to increase the ratio of oil to surfactant in a laundry solution so that the enzyme will hydrolyze oil stains. This added oil (that will be in addition to the triglyceride found on stained fabrics being washed and, together with the oily stain, constitutes the oil used as numerator in the critical ratio calculation) does not need to be a substrate for the enzyme. Use of the additional oil as a means for turning on the enzyme also allows one to remove lower levels of oily stains during laundering than would otherwise be possible.

Preferred added oils (that are not substrates) are hexadecane and octadecane. The addition of non-substrate oil is illustrated by the data of Table XI.

TABLE XI

Triolein(mM)	Hexadecane(mM)	Surfonic JL-80X(mM)	System Ratio	Enzyme Activity*
0.3	-	0.3	1	0
1.5	-	0.3	5	15
0.3	1.2	0.3	5	8

* As described by footnote 3 in Table I.

As may be seen from the data of Table XI, when triolein is at a concentration of 0.3 mM and the system ratio is 1, there is no hydrolase activity. With 1.5 mM triolein, which produces a system ratio of 5, there is hydrolase activity. When 1.2 mM hexadecane was added to the 0.3 mM triolein, then the hydrolase was found to be active in the presence of 0.3 hexadecane was added to the 0.3 mM triolein, then the hydrolase was found to be active in the presence of 0.3 mM Surfonic JL-80X surfactant even though the substrate concentration remained at 0.3 mM.

Mixtures of substrate oils can be used to manipulate the critical ratio also. Table XII demonstrates an example where the oil added _is_ a substrate and is used to increase the system ratio above the critical ratio to activate the enzyme.

TABLE XII

Trioctanoin (mM)	Triolein (m M)	Surfonic JL-80X(mM)	System Ratio	Enzyme Activity*
1.87	0	0.5	3.74	0
1.87	0.63	0.5	5	51
0	0.25	0.5	0.5	0
2.25	0	0.5	4.5	0
2.25	0.25	0.5	5	56

* % total oil hydrolyzed in 14 min. at pH 10 and room temperature.

As seen by the data of Table XII, 0.25 mM triolein, emulsified in 0.5 mM Surfonic JL-80X, is not hydrolyzed by this enzyme. Similarly, 2.25 mM trioctanoin emulsified in 0.5 mM surfonic JL-80X is also not hydrolyzed. However, when both these oils (0.25 mM triolein and 2.25 mM trioctanoin) are emulsified together in 0.5 mM surfonic JL-80X, then 56% of the total oil is hydrolyzed.

Other organic compounds which do not participate in the hydrolysis reaction (in addition to the earlier discussion of added oils such as hexadecane) can be used to reach the critical ratio. Suitable organic compounds are those that are relatively insoluble and preferably contain few to no polar groups because polar groups may interfere with enzyme activity. However, if the organic compound's polar groups are hindered or obscured by suitable branched or long chain alkyl groups, then some polarity can be tolerated. Charged substituents (e.g., $-COO^{-}Na^{+}$) are not preferred. The relatively insoluble organic compounds (which do not act as substrates for the enzyme) can be chosen, without limitation, from esters (e.g., butyl acetate, butyl butylate, butyl stearate, butyl propionate ethyl caprylate), glycol (diol) derivatives (such as diethylene glycol monolaureate, ethylene glycol dimethyl ether), alcohols (such as lauryl alcohol), aldehydes, ketones (such as methyl butyl ketone, methyl nonyl ketone), and amides (e.g., N, N-diethyldodecanamide). It is most preferred that these compounds have a solubility, δ, of between about 7-9.5, in accordance with the formula

$$\delta = \frac{\Sigma G\ d}{M}$$

where ΣG is the sum for all the atoms and groupings in the molecule, d is the density, and M, the molecular weight. Especially preferred are compounds with a solubility, δ, of between about 8.0-9.0 such as are illustrated and described by J. Brandrup and E.H. Immergut, Eds., Polymer Handbook, 2d Ed., John Wiley & Sons, 1975), pp. IV-337 to IV-353, incorporated herein by reference. It may be that these relatively insoluble organic compounds, preferably with few to no polar groups, are sufficiently chemically analogous to the oils (substrate or not) as to increase the total "effective" oil concentration. Thus, such relatively insoluble organic compounds represent another embodiment of the means for changing the ratio of oil to surfactant.

Table XIII illustrates use of a preferred, relatively insoluble, organic compound, N, N-diethyldodecanamide, to achieve the desired critical ratio when trioctanoin was the substrate.

TABLE XIII

Trioctanoin (mm)	N,N-diethyldodecanamide (mm)	Neodol 25-9 (mm)	Enzyme Activity*
2.5	--	0.5	< 5
10.0	--	0.5	35
--	10.0	0.5	< 5
2.5	2.5	0.5	5
2.5	5.0	0.5	12
2.5	7.5	0.5	19

* µeq of fatty acid produced in 14 minutes

The data of Tables XI through XIII were collected using the enzyme from *P. putida* ATCC 53552; however, other enzymes can similarly be activated even in the presence of a surfactant for which the enzyme has a high critical ratio by including an oil that is not a substrate for the enyzme in the detergent composition. This is illustrated by the data of Table XIV, where the enzyre was Amano p.

TABLE XIV

Trioctanoin (mM)	Hexadecane (mM)	Neodol 25-3S	System ratio	Enzyme Activity*
5	-	0.5	10	0
15	-	0.5	30	181
5	10	0.5	40	45

* As in footnote 3 of Table IA.

The molar ratio of oil hydrolysis activating agent (whether substrate or non-substrate) to surfactant in enzyme compositions of the invention preferably is greater than about 0.5. This is calculable from the desired critical ratio of not greater than about 1 when one assumes an average of 0.34 mM oily stains on the fabrics being laundered and an average of 0.75 mM surfactant(s).

REDUCING THE CRITICAL RATIO WITH ADDITION OF SURFACTANTS

In order to determine the critical ratio for a particular lipase or cutinase with different surfactants, the hydrolase is tested in aqueous solution for hydrolysis activity in aqueous solution with a surfactant and a hydrolyzable substrate. The ratio of surfactant and substrate is varied while hydrolysis activity is monitored. For example, Table IA illustrates the type of data that will typically be generated by varying the ratio.

Because a desired critical ratio is normally not greater than 1, one or more surfactants may need to be tested (and/or another hydrolase tested) until a critical ratio of less than or about 1 is found. For example, the enzyme tested in Table IA had a critical ratio between 0.5 - 1 when the surfactant was Neodol 25-3S and the substrate was trioctanoin. The laundering composition may then be formulated by including the lipase or cutinase and the surfactant selected to have a critical ratio of less than or about 1.

It has been discovered that mixing a surfactant with a high critical ratio for a particular enzyme with one that has a low critical ratio for that enzyme can result in a lowered critical ratio of the enzyme for the admixed surfactant. This exemplifies a means for changing the critical ratio of the surfactants so that the enzyme (of an inventive enzyme composition) will be "turned on" and hydrolysis will occur. This is illustrated by Table XV where trioctanoin was used as the oil, or substrate, and the hydrolase was as in Table II (2μg/ml). Enzyme activity was measured by initial rates and the reaction was carried out at ambient temperature to an end point pH of 10.00.

TABLE XV

Mole ratio (SDS: Neodol 25-9)	Critical Ratio
	(0.5 mM total surfactant)
0:1	10 - 20
0.025:0.975	10 - 20
0.05:0.95	5 - 10
0.1:0.9	1 - 5
0.2:0.8	1 - 3
0.5:0.5	1 - 3
0.75:0.25	1 - 3
1:0	1 - 3

Although the combination shown reduced the critical ratio to 1, this is nevertheless not low enough for the enzyme to be active. That is, as may be seen by the data of Table XV, a 0.1-0.2 mole fraction of SDS, when admixed with a high critical ratio surfactant, was effective to reduce the critical ratio of the surfactant admixture to that of SDS alone, but this is not low enough for commercial detergent formulations.

Table XVI illustrates a mixture of surfactants effective in reducing the critical ratio. Again trioctanoin was used as the oil at (0.64) mM and the hydrolase was as in Table II.

TABLE XVI

Surfactant	(mM)	System Ratio	% Total Hydrolysis
Neodol 25-9	0.3	2	0
Neodol 25-9	0.13	5	0
Neodol 25-9	0.064	10	0
Neodol 25-9	0.032	20	18
Neodol 25-9	0.016	40	47
Neodol 25-9	0.008	80	60
Neodol 25-9/ Neodol 25-3S (1:1)	5.0	0.06	26
Neodol 25-9/ Neodol 25-3S (1:1)	2.0	0.3	35
Neodol 25-9/ Neodol 25-3S (1:1)	1.0	0.6	50
Neodol 25-9/ Neodol 25-3S (1:1)	0.5	1	53
Neodol 25-9/ Neodol 25-3S (1:1)	0.3	2	60
Neodol 25-9/ Neodol 25-3S (1:1)	0.1	6	60
Neodol 25-9/ Neodol 25-3S (1:1)	0.03	21	60

As can be seen from the data of Table XVI, when Neodol 25-9 surfactant at 0.3 mM was used with 0.64 mM of the oil for a system ratio of 2, there was no hydrolysis. However, with a mixture in a 1:1 ratio of Neodol 25-9 and Neodol 35-3S surfactants for the same molar system ratio, there is 60 percent total hydrolysis. This is a surfactant system which is commercially usable.

Table XVII illustrates another example of mixing high and low critical ratio surfactants in order to reduce the critical ratio for the admixture. A surfactant composition was prepared of Neodol 25-9 -Neodol 25-3S at a constant molar ratio of 1:1. The substrate concentration (trioctanoin) was at about three times normal use levels (0.64 mM) and the percent total hydrolysis of the substrate after seven minutes was monitored as a function of changing the total surfactant concentration in the solution.

TABLE XVII

Surfactant Composition & Molar Ratio	Total Surfactant Conc.	System Ratio	Enzyme Activity**
Neodol 25-9	1.28 mM	0.5	0
Neodol 25-9 Neodol 25-3S(1:1)	1.28 mM	0.5	35
Neodol 25-9	0.64 mM	1.0	0
Neodol 25-9 Neodol 25-3S(1:1)	0.64 mM	1.0	55
Neodol 25-9	0.128 mM	5.0	0
Neodol 25-9 Neodol 25-3S(1:1)	0.128 mM	5.0	60
Neodol 25-9	0.064 mM	10.0	0
Neodol 25-9 Neodol 25-3S(1:1)	0.064 mM	10.0	60
Neodol 25-9	0.032 mM	20.0	30
Neodol 25-9 Neodol 25-3S(1:1)	0.032 mM	20.0	60

** % total oil hydrolyzed in 7 minutes.

As can be seen from the data of Table XVII, the resulting surfactant mixture showed enzyme activity at a system ratio of 0.5. Thus, the critical ratio was at least 0.5 or lower. By contrast, the Neodol 25-9 surfactant by itself had a system ratio of about 20 before enzyme activity was measured. Therefore, the inclusion of Neodol 25-3S at a 1:1 molar ratio reduced the critical ratio for Neodol 25-9 from about 20 to about 0.5.

Table XVIII illustrates another example of where a mixture of high and low critical ratio surfactants synergistically reduces the critical ratio for the admixture to a point below that for either component surfactant. A surfactant composition mixture of C_{12}LAS/Neodol 25-9 was prepared at a molar ratio of 2:1 and tested for comparison against each of the individual surfactants.

TABLE XVIII

Surfactant Composition And Concentration	Critical Ratio
C_{12}LAS (0.5 mM)	5 - 10
C_{12}LAS (5.0 mM)	1 - 5
Neodol 25-9 (0.5 mM)	10 - 20
C_{12}LAS/Neodol 25-9 (2mM/1mM)	0.05 - 0.1

In the tests illustrated by the data of Table XVIII, the surfactant mixture showed the *Pseudomonas putida* enzyme activity at a critical ratio between 0.05-0.1. The oil was triolein. By contrast, the Neodol 25-9 surfactant by itself (at 0.5 mM) had a critical ratio of between 10-20 and the critical ratio for C_{12}LAS surfactant by itself was 5-10 (at 0.5mM). Thus, the combination of these two surfactants reduced the critical ratio for the combination to a value below the critical ratio of either surfactant by itself.

COMPOSITIONS OF THE INVENTION

A preferred detergent composition, useful in unit amounts to launder fabric having a triglyceride thereon, comprises a surfactant formulation providing from about 0.2 mM to about 1.5 mM surfactant concentration when a unit amount of the total composition is dissolved in a laundry solution. Particularly preferred compositions include an enzyme isolatable from *P. putida* ATCC 53552 and in an amount sufficient to hydrolyze at least about 5 wt.% triglyceride on fabric when a unit amount of the total composition is dissolved in a laundry solution.

For example, a composition of the invention (designated "inventive composition(a)") was prepared by admixing the nonionic surfactant Neodol 23-6.5 and the nonionic surfactant Surfonic JL-80X in a 1:0.2 mole ratio. Additional additives and proportions were:

Component	wt. %
Surfactants	
(Neodol 23-6.5/	3.7
Surfonic JL-80X)	26.0
deionized water	0.6
sodium tripolyphosphate	
sodium carbonate	10.5
sodium polysilicate[1]	1.5
alkaline proteases[2,3]	0.8/0.6
brightener[4]	0.9
pigment	0.1
fragrance	0.2

[1] Trademark Britesil, available from PQ Corporation
[2] Trademark Alcalase, available from Novo Industries
[3] Trademark Savinase, available from Novo Industries
[4] Trademark Tinopal 5BM-XC, available from Ciba-Geigy A.G.

The hydrolase included in this detergent composition was grown and isolated from *P. putida* ATCC 53552 as is described in Wiersema et al., European Patent Application 268,456, published May 25, 1988, but also set out below for the reader's convenience.

(A) Seeding and Fermenting

A seed medium was prepared with 0.6% nutrient broth (Difco) and 1% glucose (pH 6.5). 100 ml of this medium was sterilized in 500 ml fernbach flasks. The flasks were each seeded with a loopful from an overnight culture of P. putida ATCC 53552 grown on nutrient agar, and placed on a New Brunswick shaker at 250 rpm, 37° C for 12 hours. The incubated 12-hour culture was then seeded at appropriate volumes (1-10% v/v) into a 1 liter fermenter (250 ml working volume), a 15 liter Biolafitte fermenter (12 liters working volume), or a 100 liter Biolafitte fermerter provided with a temperature controller, RPM, airflow and pressure controller. The fermenter medium contained 0.6% nutrient broth (Difco), 0.3% apple cutin, and 0.2% yeast extract (Difco), with an initial pH of 6.5. The medium was adjusted to pH 6.8 and sterilized for 40 minutes before seeding. Bacterial growth and enzyme production were allowed to continue in the fermenter for 12-15 hours.

(B) Enzyme Recovery bv Microfiltration

The crude fermentation culture was first filtered in a Amicon unit outfitted with two Romicon microporous membranes (0.22μ) to remove cells. Remaining enzyme in the retentate which was bound to the cutin particles was removed by centrifugation. Total recovery approached 90%.

(C) Concentration and Dialysis of Whole Cell Filtrate

The recovered filtrate from the Amicon unit was concentrated to a volume of 3 liters on an Amicon ultrafiltration unit with two Romicon Pm 10 modules. The concentrated material was then dialised with 20 liters of 0.01M phosphate buffer, pH 7.5, to remove salts and color. Recovery at this stage averaged about 80%. Total activity for this crude preparation was 8.68 x 10^6 units. A unit of lipase activity is defined as the amount of enzyme which results in an increase of absorbance at 415 nm of 1.0/minute when incubated at 20° C with mM p-nitrophenylbutyrate in 0.1 M pH 8.0 Tris-HCl buffer containing 0.1 wt.% Triton X-100.

(D) Complete Isolation of the Hydrolase

The desired enzyme may be separated completely from another enzyme also with lipase activity by chromatography on hydrophobic resins. The enzyme solution of Example III(C) after ultrafiltration and difiltration was adjusted to 0.5M NaCl and applied to a 0.8 x 7 cm octyl Sepharose column equilibrated in 10mM Tris(Cl), pH 8, 0.5M NaCl and washed to removed unbound protein. The following washes were then employed: 10mM Tris(Cl), pH 8, 7M urea; 10mM Na phosphate, pH 8; 10mM phosphate, pH 8, 0.5M NaCl. After washing, the column was then developed with a linear gradient to 50% n-propanol. The column fractions were then assayed for activity on p-nitrophenyl butyrate (PNB) and p-nitrophenyl caprylate (PNC) in order to locate the lipase activities. Two enzymes were clearly resolved, fraction 32 with a PNB/PNC ratio of 4.6 (which is the desired enzyme) and fraction 51 with a PNB/PNC ratio of 1.40.

IMPROVED OILY STAIN REMOVAL

Both swatch studies and washing machine studies were conducted with compositions of the invention as will now be described.

In the swatch study (1), 2ppm hydrolase was admixed with the detergent composition previously described as inventive composition (a). In a washing machine study (1), 20ppm hydrolase was admixed with this composition. Both studies included staining fabrics with synthetic sebum soil. The synthetic sebum soil was prepared as follows. Ten oils having the following proportions were admixed:

Oils	%w/w
Stearic acid -	5
Squalene	5
Cholesterol	5
Linoleic acid	5
Oleic acid	10
Paraffin oil	10
Palmitic acid	10
Coconut oil	15
Sperm wax	15
Olive oil	20

To 15g of the above melted oils was added 0.6 g oleic acid, 1.2 g triethanolamine and 0.225 g charcoal. Then 60 ml water at 130° F was admixed, and the mixture blended for 1 minute.

Swatch study (1)

Cotton swatches were stained with the synthetic sebum soil and then washed in test beakers by agitating for 14 minutes followed by a 5 minute rinse. The laundering solution was 0.205g of inventive composition(a) dissolved in 250ml water. A control composition without the hydrolase was also prepared and used to treat stained cotton swatches by the same protocol. Table XIX shows the stain removal for the inventive composition (a) and for the control composition.

TABLE XIX

Composition	System Ratio [1]	%SR(E) [2]
Inventive comp.(a)	0.08	60.72
Control	0.08	57.37

[1] Calculated from concentrations of surfactant and the olive and coconut oils.
[2] LSD = 2.23 at 0.95 confidence level.

As may be seen from the swatch study data of Table XIX, statistical enhancement of soil removal was seen for the inventive composition.

Washing Machine Study (1)

Polyester swatches were stained with sebum, vegetable oil or olive oil. These swatches were then washed for 12 minutes at 96 F in a 72 liter washing machine, rinsed in the normal rinse cycle and then allowed to air dry. One set of swatches was treated in laundering solution having 59 g inventive composition(a) dissolved therein while another set of swatches was treated with a control composition identical to inventive composition(a) but without the hydrolase. The stain removal data, expressed as %SR-(E), is shown by Table XX.

TABLE XX

Composition	Sebum [1]	Vegetable Oil [2]	Olive Oil [3]
Inventive comp.(a)	89.69	51.82	60.79
Control	83.75	29.20	35.05

[1] LSD = 1.52
[2] LSD = 6.08 (Wesson brand oil)
[3] LSD = 5.60

As can be seen from the data of Table XX, statistically significant stain removal was achieved for all stains tested on polyester fabric.

Swatch and Washing Machine Study (2)

The polycotton fabric was cut into 2" x 2" swatches, each weighing about 0.39 g. The desired amount of triolein was dissolved in 2-methyl pentane, and pipetted onto each swatch (200 μL/swatch). The triolein stain was allowed to wick out for 72 hrs. at room temperature. The reflection of the stain was then evaluated using a Hunter Spectracolorimeter, and a prewash value (proportional to the concentration of the absorbing species) was determined.

The soiled swatches were divided into groups of 4 and loaded into 250 ml bottles, each with 200 ml of the desired treatment. The bottles were then shaken for 12 minutes at room temperature, and rinsed twice with 200 ml of dd H_2O. Finally they were air dried and the postwash value (proportional to the concentration of the absorbing species) determined.

Comparative Treatment A: The swatches were washed in a surfactant composition of 0.3 mM C_{12}LAS/Neodol 25-9 in a 2:1 molar ratio. No lipase was added.

Inventive Treatment B: Same as A, except for the addition of a 5 ppm lipase ATCC 53552 to the surfactant composition.

Comparative Treatement C: The swatches were washed in an alternate formulae containing the surfactant composition of 0.3 mM C_{12}LAS/Neodol 25-9, in a molar ratio of about 1:4. No lipase was added.

Inventive Treatment D: Same as treatment C, except for the addition of 5 ppm lipase ATCC 53552 to the surfactant composition.

The amount of oily stain removed in each treatment is summarized by Table XXI.

TABLE XXI

Oil Loaded		System Ratio	Treatment	% Triolein Removal***	LSD
%*	mM**				
1	0.072	0.24	A (comparative)	44	3.8
1	0.072	0.24	B (inventive)	55	
3	0.22	0.73	A (comparative)	29	3.7
3	0.22	0.73	B (inventive)	34	
1	0.072	0.25	C (comparative)	39	4.5
1	0.072	0.25	D (inventive)	60	
3	0.22	0.76	C (comparative)	27	3.6
3	0.22	0.76	D (inventive)	33	

*% Triolein loaded reflects the grams of oil per 100 g of fabric.

** The mM concentrations were calculated based on bulk concentrations of oil and surfactants.

*** % removal was calculated by comparing the pre and post wash values to a standard curve vs oil on swatch.

As may be seen by the data of Table XXI, use of a composition in accordance with the invention removed from 33% to 60% of the oil on the polycotton fabric, and this removal was distinctly better for the inventive composition (including the hydrolase) than without hydrolase. The LSD values show this removal was statistically significant.

In sum, enzyme compositions of the invention are useful in laundering solutions and comprise an enzyme capable of hydrolyzing natural oil stains on fabric when in a laundry solution and hydrolysis activating means for changing the ratio of oil to surfactant or for changing the critical ratio of the surfactants. Several embodiments of hydrolysis activating agents have been exemplified for use in laundry solutions so that the enzyme will be active in hydrolyzing the oil stains. One can usually observe the onset of hydrolysis when the enzyme has an activity sufficient to hydrolyze at least about 5 wt. % of total triglyceride stains within about 14 or 15 minutes at about 25° C. Without the hydrolysis activating means of the invention, the enzyme is normally inhibited from hydrolyzing natural oily soils or stains when the laundering solution contains between about 0.1 mM to 5 mM of surfactant. Another way of stating the effect of the hydrolysis activating means of the invention is that when a lipase or cutinase is admixed with a surfactant formulation in accordance with this invention, then the lipase or cutinase is capable of hydrolyzing at least about 30 mg triolein when a unit amount of laundering composition is dissolved in aqueous solution at 25° C at pH 10 with an average rate of about 0.0072 mmoles/min fatty acid being produced over about 14 minutes. Thus, surfactant systems may be formulated in accordance with the invention that include lipases and/or cutinases for use in laundering solutions without requiring extended soaking or high temperatures.

Although the present invention has been described with reference to specific examples, it should be understood that various modifications and variations can be easily made by those skilled in the art without departing from the spirit of the invention. Accordingly, the foregoing disclosure should be interpreted as illustrative only and not to be interpreted in a limiting sense. The present invention is limited only by the scope of the following claims.

Claims

1. An enzyme composition for laundry applications comprising:
an enzyme capable of hydrolyzing natural oil stains on fabric when in a laundry solution, the onset of

hydrolysis being dependent upon a critical value of the molar ratio of stain oil to surfactant in the laundry solution, said critical ratio being dependent upon the surfactant used; and

hydrolysis activating means for increasing the ratio of oil to surfactant in a laundry solution in which the composition is employed so that the enzyme will hydrolyze oil stains.

2. The enyme composition as in claim 1 wherein the hydrolysis activating means includes a hydrocarbon, a substrate for the enzyme, a relatively insoluble organic compound, or mixtures thereof.

3. The enzyme composition as in claim 2 wherein the hydrolysis activating means includes hexadecane, octadecane, trioctanoin, triolein or N, N-diethyldodecanamide.

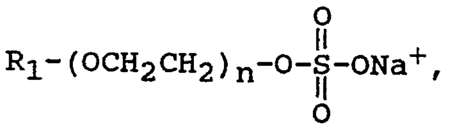
4. The enzyme composition as in claim 1 wherein the enzyme is a lipase or cutinase.

5. The composition as in claim 4 wherein the enzyme is isolatable from an organism expressing a gene obtainable from a *Pseudomonas*, a *Chromobacter*, an *Aspergillus*, an *Acinetobacter*, or a *Fusarium*.

6. The enzyme composition as in claim 4 wherein the enzyme is isolatable from Pseudomonas putida ATCC 53552, mutants thereof or clones thereof.

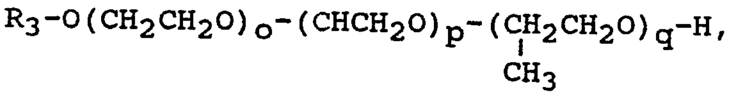
7. The enzyme composition as claimed in any of claims 1-6 further comprising at least one surfactant, the molar ratio of said oil hydrolysis activating agent to said surfactant being greater than about 0.5.

8. The enzyme composition as in claim 7 wherein the surfactant is selected from the group consisting of sodium dodecylsulfate, $C_{12}H_{25}$-ø-SO_3^- Na^+,



$$R_1-(OCH_2CH_2)_n-O-\underset{\underset{\displaystyle O}{\|}}{\overset{\overset{\displaystyle O}{\|}}{S}}-ONa^+,$$

$$R_2-(OCH_2CH_2)_m-H,$$

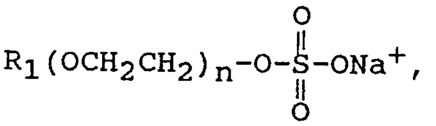


$$R_3-O(CH_2CH_2O)_o-(CHCH_2O)_p-(\underset{\underset{\displaystyle CH_3}{|}}{CH_2}CH_2O)_q-H,$$

C_8H_{17}-ø$(OCH_2CH_2)_r$-H,

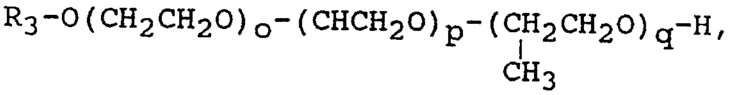
and mixtures thereof, where R_1 is an alkyl having about 10 to 15 carbons, or a mixture thereof, and n averages about 3, R_2 is an alkyl having about 12 to 15 carbons, or a mixture thereof, and m averages about 9, R_3 is an alkyl having about 9 to 11 carbons, or a mixture thereof, and o averages about 4.5, p averages about 1.5 and q averages about 4.5, and where r averages about 10.

9. The composition of claim 8 wherein the surfactant is selected from the group consisting of sodium dodecylsulfate, $C_{12}H_{25}$-ø-SO_3^- Na^+,



$$R_1(OCH_2CH_2)_n-O-\underset{\underset{\displaystyle O}{\|}}{\overset{\overset{\displaystyle O}{\|}}{S}}-ONa^+,$$

$$R_2(OCH_2CH_2)_m-H,$$



$$R_3-O(CH_2CH_2O)_o-(CHCH_2O)_p-(\underset{\underset{\displaystyle CH_3}{|}}{CH_2}CH_2O)_q-H,$$

and mixtures thereof, where R_1 is an alkyl having about 10 to 15 carbons, or a mixture thereof, and n averages about 3, R_2 is an alkyl having about 12 to 15 carbons, or a mixture thereof, and m averages about 9, R_3 is an alkyl having about 9 to 11 carbons, or a mixture thereof, and o averages about 4.5, p averages about 1.5 and q averages about 4.5.

10. A method of laundering fabrics having oily stains thereon in a solution containing a lipase or a cutinase and at least one surfactant comprising:

adding to the solution an hydrolyzing activator comprising an agent selected from the group consisting of a hydrocarbon, a substrate for the enzyme, a relatively insoluble organic compound, and mixtures thereof.

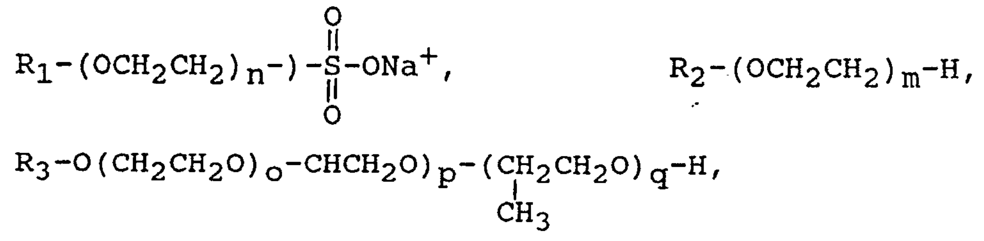
11. A method of laundering fabrics having oily stains thereon in a laundry solution comprising:

adding to said solution at least one surfactant, a lipase or cutinase, and an hydrolyzing activator selected from the group consisting of a hydrocarbon, a substrate for the enzyme, a relatively insoluble organic compound, and mixtures thereof.

12. A method as claimed in claim 10 wherein surfactant and activator are added to the solution so that the molar ratio of activator and surfactant is above the critical ratio of the enzyme for said surfactant, the molar ratio of activator to surfactant preferably being greater than about 0.5.

13. A method as claimed in claim 11 or claim 12 wherein the enzyme is isolatable from an organism expressing a gene obtainable from a Pseudomonas, a Chromobacter, an Aspergillus, an Acinetobacter, or a Fusarium, and is preferably isolatable from Pseudomonas putida ATCC 53552, mutants thereof or clones thereof.

14. A method as claimed in any of claims 11-13 wherein the surfactant is selected from the group consisting of sodium dodecylsulfate, $C_{12}H_{25}$-ø-SO_3^- Na^+,



$$R_1-(OCH_2CH_2)_n-)-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}-ONa^+, \qquad R_2-(OCH_2CH_2)_m-H,$$

$$R_3-O(CH_2CH_2O)_o-CHCH_2O)_p-(\underset{CH_3}{\underset{|}{C}}H_2CH_2O)_q-H,$$

and mixtures thereof, where R_1 is an alkyl having about 10 to 15 carbons, or a mixture thereof, and n averages about 3, R_2 is an alkyl having about 12 to 15 carbons, or a mixture thereof, and m averages about 9, R_3 is an alkyl having about 9 to 11 carbons, or a mixture thereof, and o averages about 4.5, p averages about 1.5 and q averages about 4.5.

15. An enzyme composition for laundry applications comprising:
an enzyme capable of hydrolyzing natural oil stains on fabric when in a laundry solution, the onset of hydrolysis being dependent upon the molar ratio of oil to surfactant in the laundry solution exceeding a critical value, said critical ratio being dependent upon the specific surfactant; and
hydrolysis activating means for changing the critical ratio of the surfactant in a laundry solution in which the composition is employed so that the enzyme will hydrolyze the oil stains.

16. The composition as in claim 15 wherein the activating means comprises a surfactant, said surfactant being selected whereby when in combination with other surfactant in the laundry solution the critical ratio of the combination will be not greater than about 1.

17. The composition of claim 15 or 16 further comprising at least one surfactant having a first critical ratio with respect to the enzyme, and wherein said activating means comprises a second surfactant having a second critical ratio with respect to the enzyme, said surfactants being selected whereby the critical ratio of the combination of the surfactants is at or below the second critical ratio.

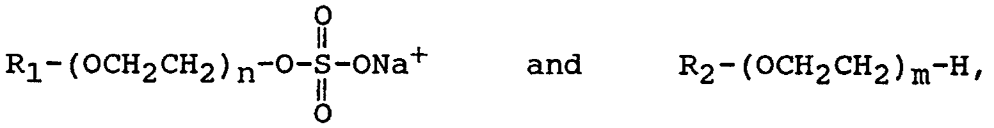
18. The composition as claimed in any of claims 15-17 wherein the enzyme is isolatable from Pseudomonas putida ATCC 53552, mutants thereof or clones thereof.

19. The composition as claimed in any of claims 15-18 wherein the surfactants are

$$R_1-(OCH_2CH_2)_n-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}-ONa^+ \quad \text{and} \quad R_2-(OCH_2CH_2)_m-H,$$

where R_1 is an alkyl having about 10 to 15 carbons, or a mixture thereof, and n averages about 3, and R_2 is an alkyl having about 12 to 15 carbons, or a mixture thereof, and m averages about 9.

20. The composition of claim 19 wherein the surfactants are



$$R_1-(OCH_2CH_2)_n-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}-ONa^+ \quad \text{and} \quad R_2-(OCH_2CH_2)_m-H,$$

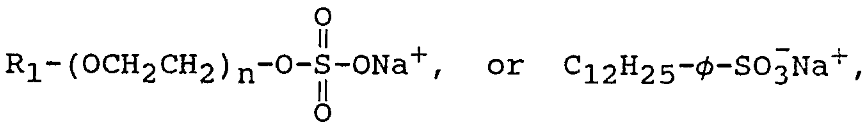
where R_1 is an alkyl having about 10 to 15 carbons, or a mixture thereof, and n averages about 3, and R_2 is an alkyl having about 12 to 15 carbons, or a mixture thereof, and m averages about 9.

21. A method of effectively using a lipase or cutinase in a surfactant laundry solution to hydrolyze oily stains on fabric where the surfactant in the solution will inhibit hydrolysis of oily stains by the enzyme when the molar ratio of fabric stain oil to recommended use levels of surfactant is below the critical ratio for such

enzyme and surfactant, comprising:
adding to the surfactant laundry solution a lipase, and an hydrolysis activating surfactant, said hydrolysis activating surfactant being selected so that the critical ratio of the resulting combination of surfactants is equal to or less than that of the hydrolysis activating surfactant.

22. The method as claimed in claim 21 wherein the enzyme is isolatable from Pseudomonas putida ATCC 53552, mutants thereof or clones thereof.

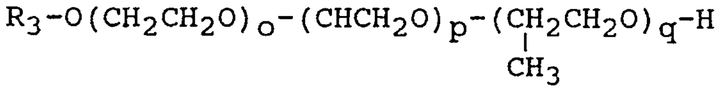
23. The method as claimed in claim 21 or 22 wherein the hydrolysis activating surfactant is sodium dodecylsulfate,



$$R_1-(OCH_2CH_2)_n-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}-ONa^+, \quad \text{or} \quad C_{12}H_{25}-\phi-SO_3^-Na^+,$$

and mixtures thereof, where R_1 is an alkyl having about 10 to 15 carbons, or a mixture thereof, and n averages about 3.

24. The method as claimed in claim 21 or claim 22 wherein the surfactants are



$$R_3-O(CH_2CH_2O)_o-(CHCH_2O)_p-(\underset{\underset{CH_3}{|}}{C}H_2CH_2O)_q-H$$

or $R_4-(OCH_2CH_2)_s-H$, where R_3 is an alkyl having about 9 to 11 carbons, or a mixture thereof, and o averages about 4.5, p averages about 1.5 and q averages about 4.5, and R_4 is an alkyl having about 12 to 13 carbons and s averages about 6.5.

25. A method for selecting a surfactant system, useful in a laundering solution, with which a lipase or cutinase is active, comprising:

(a) providing, in an aqueous solution, a lipase or a cutinase, a first surfactant and a first oil, the first surfactant and first oil defining a first molar ratio of oil to surfactant, the first oil being a hydrolyzable substrate for the lipase or cutinase;

(b) manipulating the molar proportions of surfactant and oil in a manner such that a critical ratio is attained, resulting in hydrolysis of said first oil; and

(c) determining thereby a laundering composition including the lipase or cutinase and the selected surfactant or surfactant mixture.