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Enzymes in Detergents[1]

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Abstract

Factors affecting the performance of proteolytic and amylolytic enzymes in an anionic and nonionic detergent formulation have been studied using stain removal from EMPA blood-milk-ink and cocoa-milk-sugar soil test cloths as a measure of enzyme activity in the detergent solution. Factors considered include enzyme concentration, and temperature and pH of the wash solution. Results on stability of these enzymes in the two detergent formulations under accelerated storage conditions are also given.

Introduction

In recent months there has been a number of papers and articles in technical journals and trade magazines on enzymes as related to detergent products (1–6). In fact, their number closely parallels the large number of enzyme-containing detergent products currently in proprietary markets or test markets (6). Why the sudden popularity of enzymes and enzyme products? It appears that with the incorporation of enzymatic activity into household detergent products the housewife is able to see a demonstratable performance benefit in the removal of certain formerly stubborn stains and soils (7). Many housewives ascribe a more delicate cleaning operation to the new "easy care" fabrics and garments: this has made her more conscious of a need for yet another product in her arsenal of cleaning materials. With the new enzyme products she apparently visualizes a subtle, gentle, specific and safe removal of soils and stains without resort to more harsh treatment. Further, a widespread success experience in Europe has given domestic detergent producers the background and confidence for a rapid and aggressive product introduction program in the United States.

[1] Presented at the AOCS Meeting, New York, October, 1968.

TABLE I

Activity Values for Enzymes Considered in This Study

Enzyme	Units/gram of enzyme	
	Enzyme A	Enzyme B
Protease at pH 10.3	330,000	320,000
Protease at pH 7.0	200,000	1,340,000
α-Amylase at pH 6.0	7,000	310,000

In simple terms, an enzyme can be defined as a catalyst produced by living cells. While the spectrum of reactions catalyzed by enzymes is very broad, the catalytic action of an enzyme is usually quite specific. Enzymatic reactions under optimum conditions are very rapid and efficient, proceeding 10^8 to 10^{11} times more rapidly than the corresponding nonenzymatic reaction. Enzymes are complex proteins of high molecular weights consisting of hundreds of amino acids combined in a characteristic sterically oriented structure. This structure may contain metal ions and other linking agents to provide its tridimensional steric pattern. Further, appended to these complex structures are many reactive groups which are quite vulnerable to modification by hydrolysis, heat, pH, ionic effects, sequestration, oxidation and most of other types of chemical reactions. Since this is true, it is important to know the effects of the various laundry conditions and detergent compositions on

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TABLE II
Composition of Detergents Used to Study Enzyme Performance

Ingredient	Per Cent by Weight	
	Anionic	Nonionic
Sodium tripolyphosphate	40.0	40.0
Alkylbenzene sulfonate	18.0	
Alcohol ethoxylate	....	10.0
Sodium metasilicate	6.0	6.0
Carboxymethylcellulose	0.7	0.7
Optical brighteners	0.4	0.4
Sodium sulfate	26.9	34.9
Water	8.0	8.0

these enzymes, so that maximum benefits can be obtained from their use. This paper presents the results of studies to determine the effect of certain of these factors on proteolytic and amylolytic enzyme performance.

Materials and Methods

Enzymes Used in Study

Protease enzymes used in detergent products are produced generally in fermentation processes with a *Bacillus subtilis* organism. The two protease enzymes used in these studies were produced from this type of organism. Assay data based on a modified Kunitz casein method for protease activity and a starch digestion method for α-amylase are given in Table I. Enzyme A is a typical alkaline protease with 330,000 casein units per gram when assayed at an alkaline pH of 10.3. The lower activity of 200,000 units/g at pH 7 indicates the absence of any neutral protease in the enzyme. The low α-amylase value is typical of alkaline protease enzymes of this class. Enzyme B differs from Enzyme A in that it contains neutral protease and a higher level of α-amylase in addition to approximately the same amount of alkaline protease. The neutral protease activity of this enzyme can be approximated by subtracting the alkaline protease activity observed at pH 10.3 from the total protease activity of the enzyme at pH 7. Hence, for Enzyme B the neutral protease activity is approximately 1,000,000 casein units per gram.

Detergent Compositions

The compositions of the anionic and nonionic detergents used in this study are given in Table II. These represent typical laundry detergent formulations for each of these actives. The anionic active was an average C_{13} linear alkylbenzene sulfonate and the nonionic was a C_{14-15} linear alcohol with 12 moles of ethylene oxide.

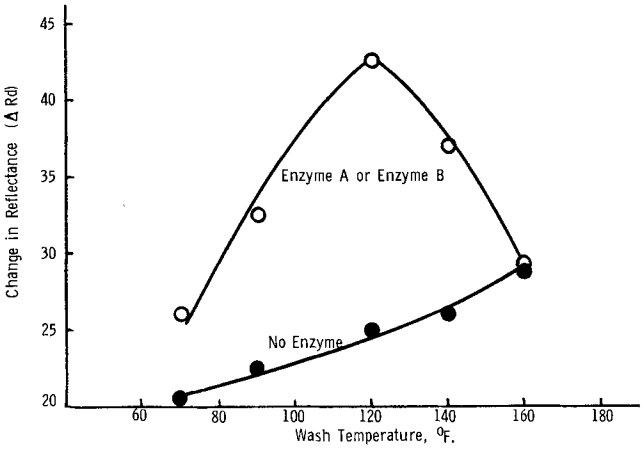

FIG. 1. Effect of temperature on stain removal from EMPA blood-milk-ink stain.

Method for Determining Enzyme Performance

The most commonly used standard stains for evaluating the functional performance of enzymes in detergent products are the EMPA blood-milk-ink and cocoa-milk-sugar soil test cloths. The blood-milk-ink soil is used to evaluate proteolytic enzymes, whereas the cocoa-milk-sugar stain is responsive to carbohydrase or amylolytic activity. These standard stains, like the standard soil cloths used by many laboratories for detergency testing, do not necessarily give practical results, but rather are used primarily for screening purposes to indicate enzyme activity in a detergent solution. They have been used in this paper to illustrate the effect of washing and formulation variables on enzyme performance.

To determine the effect of the variables considered in this study the EMPA-stained fabric was washed in solutions containing the detergent under investigation both with and without the enzyme. The difference in results then is the performance attributable to the enzyme. The specific conditions used in these tests were the following: Terg-O-Tometer speed, 90 rpm; volume wash solution, 1 liter; wash time, 10 min; temperature, 120 F; water hardness (3/2 Ca/mg), 150 ppm; detergent concentration, 1.5 g/liter; enzyme concentration, 1100 casein units/liter or 1250 α-amylase units/liter. Stained fabric characteristics were: EMPA No. 116 or No. 112, 3 × 4½ in. Four swatches were used per wash. These conditions were held constant unless they were one of the variables under study, in which case the values are so indicated.

TABLE III
Effect of Protease or α-Amylase Concentration on Stain Removal From EMPA-Stained Fabric

Enzyme concentration	Stain removal, ΔRd units							
	Anionic formulation				Nonionic formulation			
	Enzyme A		Enzyme B		Enzyme A		Enzyme B	
	Total ΔRd	ΔRd due to enzyme	Total ΔRd	ΔRd due to enzyme	Total ΔRd	ΔRd due to enzyme	Total ΔRd	ΔRd due to enzyme
Alkaline protease casein units/liter water								
0	28	0	28	0	25	0	25	0
550	38	10	37	9	36	11	35	10
1100	42	14	42	14	39	14	40	15
2200	44	16	44	16	41	16	41	16
3300	45	17	45	17	42	17	43	18
α-Amylase starch units/liter water								
0	12	0	12	0	7	0	7	0
500	14	2	22	10	8	1	16	9
1250	17	5	26	14	11	4	20	13
2500	18	6	28	16	12	5	24	17
3750	....	....	30	18	....	....	26	19

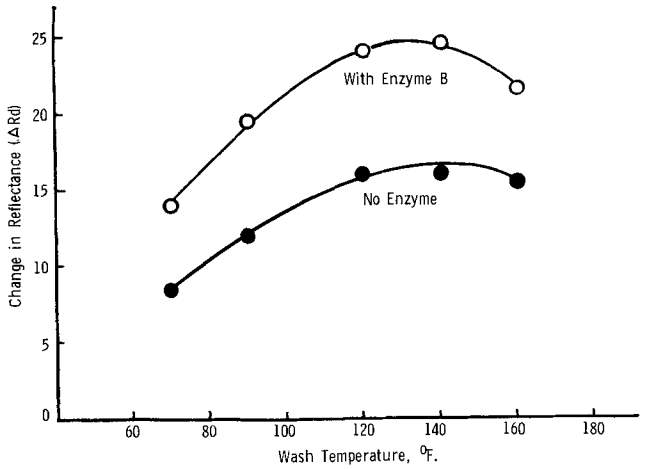

FIG. 2. Effect of temperature on stain removal from EMPA cocoa-milk-sugar stain.

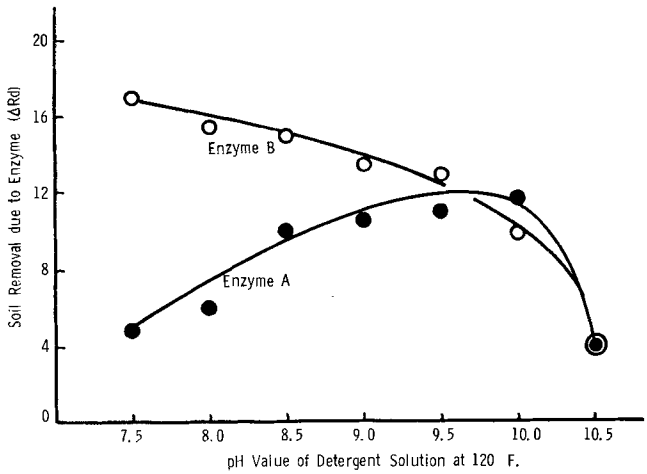

FIG. 4. Effect of pH value of detergent solution on performance of Enzyme A and Enzyme B on EMPA blood-milk-ink stain.

Performance Results

Enzyme Activity

The relationship between proteolytic activity or amylolytic activity per liter of wash water and stain removal (Δ Rd) from the EMPA-stained fabrics is shown in Table III for both the anionic and nonionic detergent formulations. The first column of numbers for each enzyme gives the total stain removal for the detergent and the protease or amylase concentration, i.e., the stain removal due to detergent plus enzyme activity. Considering these results one finds better results are obtained with the anionic formulations for both kinds of enzymes. When the detergent effect (zero enzyme concentration) is subtracted from the total Δ Rd, we find the stain removal is essentially the same at any protease or amylase concentration for the two types of detergents. This means that any effect of the surfactant on the enzyme under these wash conditions is the same in each case. Enzyme A, which has a low level of α-amylase compared to Enzyme B, gave a much poorer performance than would be expected when compared to Enzyme B at an equivalent α-amylase activity.

Wash Temperature

The effect of wash temperature on EMPA blood-milk-ink stain removal performance of Enzyme A or Enzyme B in an anionic detergent formulation is shown in Figure 1. Both enzymes had the same performance under these conditions. These data show a large temperature dependency for the protease enzyme. The greatest benefit from the protease enzyme is realized at wash temperatures around 120 F, which is near the average wash temperature used in the home. At cold water wash temperatures (60–70 F), the enzyme produces only slightly better stain removal than the detergent alone. Wash temperatures higher than 120 F cause degradation of the enzyme in the presence of the detergent as indicated by the fall-off in enzyme performance. The coincidence of the data points at 160 F indicates that this is the wash temperature at which there is essentially complete inactivation of the enzyme.

The effect of wash temperatures on EMPA cocoa-milk-sugar stain removal performance of an anionic formulation with and without Enzyme B is given in Figure 2. These curves show, first of all, that removal of the cocoa-milk-sugar stain improves with increasing temperatures up to 120 F. However, the amount of stain removal, primarily attributable to α-amylase activity, is nearly the same over the temperature range from 70–140 F. At 160 F, there is evidence of some α-amylase degradation. Comparing these results with those in Figure 1 on alkaline protease, we find the α-amylase activity is more stable at the higher wash temperatures than alkaline protease activity and less temperature-dependent.

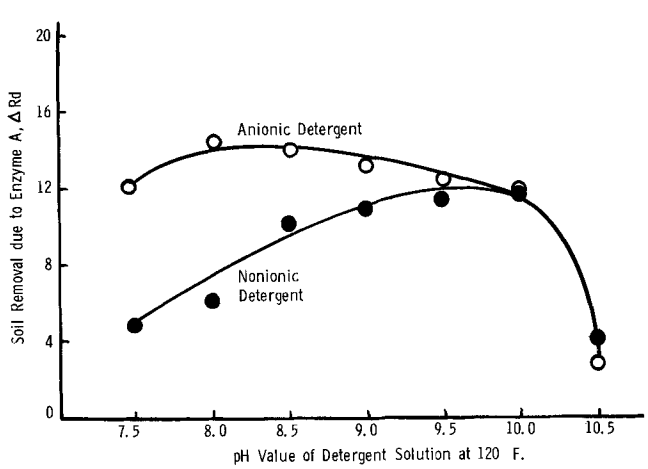

FIG. 3. Effect of pH value of detergent solution on Enzyme A performance on EMPA blood-milk-ink stain.

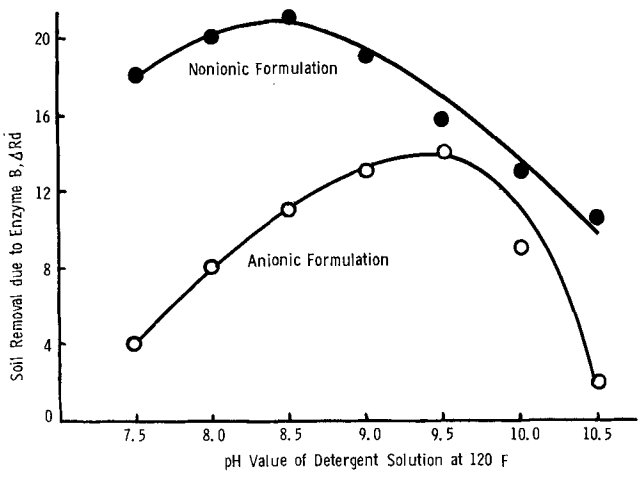

FIG. 5. Effect of pH value of detergent solution on Enzyme B performance on EMPA cocoa-milk-sugar stain.

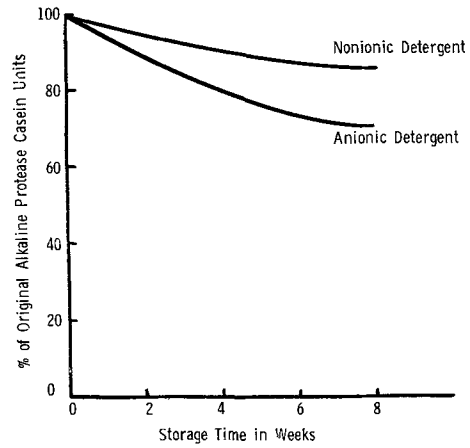

FIG. 6. Storage stability of Enzyme A in an anionic and nonionic detergent formulation.

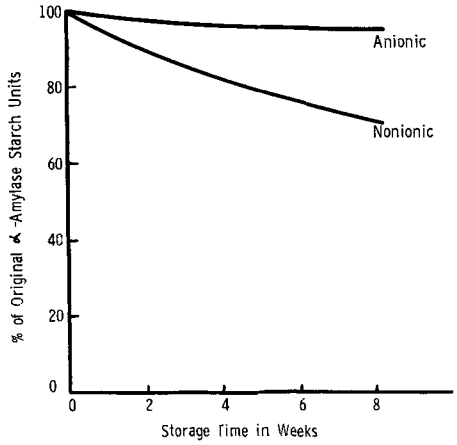

FIG. 7. Storage stability of α-amylase in Enzyme B in an anionic and nonionic detergent formulation.

Wash Solution pH

The effect of pH on the performance of Enzyme A, an alkaline protease, in an anionic and nonionic detergent formulation is shown in Figure 3. The pH of the detergent solution was adjusted with either sodium hydroxide or sulfuric acid to obtain these data. They show that the enzyme has an apparent greater tolerance to pH in the presence of an anionic detergent. In both instances, the stain removal performance falls off quickly and substantially at pH values greater than 10.

An example of the difference in enzyme performance between an alkaline protease (Enzyme A) and an enzyme containing both an alkaline and neutral protease (Enzyme B) at different pH values is given in Figure 4. The data show similar performance for the two enzymes in the pH range of 9.5–10.5, but at lower wash pH values the enzyme containing the neutral protease outperforms the alkaline protease by a large margin.

Figure 5 shows the effect of pH on the α-amylase performance in Enzyme B in both an anionic and nonionic detergent. These data show that a greater performance is obtained from the α-amylase in the nonionic detergent formulation than in the anionic formulation over the pH range of 7.5–10.5. The maximum α-amylase performance is obtained at a pH of 8.5 for the nonionic detergent and pH 9.5 for the anionic detergent. Furthermore, the data illustrate that the α-amylase is almost completely inactivated at a pH of 7.5 in an anionic detergent. This is a surprising result considering that the maximum activity of the α-amylase in the absence of detergent ingredients occurs at a pH value of about 6. Thus, one may conclude that the LAS surfactant in this formulation may have an inactivation or inhibition effect on α-amylase at pH values below about 9.

Storage Stability

The storage stability of Enzyme A, an alkaline protease, in an anionic and nonionic detergent formulation stored under accelerated conditions of temperature (90 F) and relative humidity (85%) is given in Figure 6. The enzyme was admixed with the detergents to give 1500 casein units of proteolytic activity per gram of detergent. Results of these tests show the alkaline protease to be slightly more stable in the nonionic formulation. However, the loss of proteolytic activity in both cases is not great under the highly exaggerated and aggressive storage conditions, suggesting there should be no problem with stability under ambient storage conditions.

The storage stability of the α-amylase portion of Enzyme B in an anionic and nonionic detergent formulation stored under accelerated conditions of temperature and relative humidity is shown in Figure 7. The enzyme was dry mixed with the detergents to give 1600 units of α-amylase activity per gram of detergent. The data show the α-amylase to be more stable in the presence of the anionic detergent, although the loss of activity is small in either case for such aggressive storage conditions.

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