EFFECT OF ENZYME ON DETERGENCY IN NON-AQUEOUS SYSTEM

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ABSTRACT: The effect of protease on detergency of surfactant (AOT)/isooctane/water system was studied. For evaluating the detergency of the system, the artificially blood-soiled fabric, EMPA-111, was used. The enzyme exhibited pronounced activity in the system. The enzyme would be solubilized in the core of reverse micelle formed. The solubilized water, which may be in the reverse micelle in the system, was essential for the enzyme action. In the system with low water content, \( w = [H_2O]/[AOT] < 20 \), the effect of enzyme was less remarkable. The effect of enzyme was distinguishing in the system at low surfactant concentration. In the system the enzyme considerably enhanced the detergency, while the system without enzyme did not show significant detergency. With increasing the surfactant concentration in the system, the detergency of the system with or even without enzyme increased. The contribution of enzyme, therefore, became less remarkable at high surfactant concentrations.

1. INTRODUCTION

Recently, enzymes, such as protease, lipase or cellulase, have been often used to assist the detergency in aqueous detergent system. Most of detergents (80% or more) for the domestic use contain enzymes. The effect of the enzyme on detergency in aqueous system has been widely studied.\(^1\) It is considered that enzyme, protease or lipase, in detergent system hydrolyzes proteinic or lipidic soil on fabric, respectively, to smaller molecules which can be easily solubilized or dispersed into the detergent solution. Cellulase is considered to hydrolyze microfibril of cellulosic fiber substrate and assist to dig up soils which bound firmly deep in cellulosic fiber.

Non-aqueous detergent system has also been widely applied especially to "dry-cleaning" to wash water-sensitive clothes made of wool or silk. A few examples of the utilization of enzyme to the system have been presented,\(^2,3\) but the behavior of the enzymes in the non-aqueous system has not been known in detail. Surfactant in non-aqueous medium forms reverse micelle in which polar soil as well as water molecules are solubilized. Enzyme would also be solubilized in the core of reverse micelle, protected from the non-aqueous medium.\(^4\) If the enzyme in non-aqueous system is active as well as in the normal aqueous system, it could be applied for practical use in the similar manner to that of aqueous system.

In this paper, the effect of protease on detergency of Aerosol-OT (AOT: di-2-ethylhexyl sodium sulfosuccinate)/isooctane/water system for the artificially blood-soiled fabric has been studied.

2. EXPERIMENTAL

Enzyme used was NAGARSE (Crystallized Lyophilized Bacterial Al-Proteinase, \( 114 \times 10^4 \) PUN/g) which was kindly supplied by Nagase Biochemical Ltd. Isooctane (Nacalai Tesque, guaranteed grade) and Aerosol-OT (AOT, Nacalai Tesque) were used without further purification. The artificially blood-soiled fabric, EMPA-111 (Eidgenössische Materialprüfungs und Versuchsanstalt für Industrie, Bauwesen und Gewerbe) was used to evaluate the detergency of each system. The enzyme solubilized in AOT/isooctane/water was buffered with sodium phosphate buffer at pH 7.0.

The detergency test of each system was done by washing 3 × 3 cm (0.2 g) of a blood-soiled swatch with 25 ml of each detergent system at 40°C for 60 min, and the reflectance of the sample was then measured with spectrophotometer (Shimadzu MPS-2000 color pack system). The soil removal, SR (%), was evaluated from approximate Eq. (1) by means of K/S...
(Kubelka-Munk) value derived from Eq. (2),
\[
SR(\%) = \frac{1 - (K/S)_w}{(K/S)_s} \times 100
\]
where \((K/S)_s\) and \((K/S)_w\) are K/S values of the swatch before and after washing, respectively.
\[
K/S = \frac{(1 - R)^2}{2R}
\]
where \(R\) is the reflectance of the soiled fabric at 500 nm.

3. RESULTS AND DISCUSSION

Fig. 1 shows the effect of the enzyme on detergency for blood-soiled fabric in AOT/isoctane/water system. The soil removal of AOT/isoctane/water/enzyme system for blood-soiled fabric has been plotted against the logarithm of enzyme concentration. AOT concentration in iso-octane is 0.03 mol/l, the molar ratio of water solubilized to AOT, \(w = [H_2O]/[AOT]\), is 30. As can be seen in Fig. 1, with increasing enzyme concentration in the system the removal of blood soil increases significantly, whereas the same system without the enzyme, shown by broken line in the figure, does not show clear effect. This indicates that the enzyme exhibits pronounced activity also in non-aqueous medium, not only in aqueous system, and enhances the detergency of the system by hydrolyzing the proteinic soil on the fabric.

Fig. 2 shows the effect of solubilized water in the system on detergency. The soil removal of AOT/isoctane/water system with and without enzyme are plotted against the amount of solubilized water, \(w\)-value. Concentrations of AOT and enzyme in isoctane are 0.1 mol/l and 700 PUN/l, respectively. As can be seen in Fig. 2, the detergency of the system with or without enzyme in the range of \(w\)-value from 5 to 20 do not show clear differences. Above \(w = 20\), however, the effect of the enzyme become significant. This indicates that enough amount of solubilized water is essential for the enzyme action in non-aqueous detergent system.

Luisi et al.5) pointed out that the enzyme might be solubilized in the core of reverse micelle of the surfactant in non-aqueous surfactant solution. The solubilization of water to the system may enlarge the size of reverse micelle6) and, accordingly, may dilute the ionic density in the micelle. Therefore, the fact that
the more solubilized water is in reverse micelle, the more active the enzyme in the system may be because of the less inhibition by the surfactant ions. In addition, the larger the size of the micelle is, the more the hydrolyzed proteinic soil could be solubilized.

Fig. 3 illustrates the effect of surfactant concentration on detergency of the AOT/isooctane/water system with and without enzyme. Soil removal of each system has been plotted versus logarithm of surfactant concentration. Concentration of enzyme and amount of water solubilized, (w-value) are 700 PUN/l and 30, respectively. Fig. 3 shows that at low AOT concentration of 0.03 mol/l, the system without enzyme does not show any detergency. On the other hand, the same system with enzyme (700 PUN/l) shows significant detergency (ca. 30 %). With increasing the AOT concentration, the soil removal of the system, even without enzyme, increases up to 60 % which is close to that of the same system with enzyme. The effect of enzyme is less remarkable at high AOT concentration. Even by a system without enzyme the blood soil could be removed if the system contained a large amount of surfactant. Nevertheless, it should be emphasized that in the system with enzyme, 1/3 amount of AOT is necessary to obtain high detergency compared to the system without enzyme. The utilization of enzyme in non-aqueous detergent system can reduce the surfactant concentration without lowering the detergency.

REFERENCES
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